A STUDY ON BIOFILM FORMATION IN ORGANISMS CAUSING CENTRAL VENOUS CATHETER RELATED BLOOD STREAM INFECTION IN INTENSIVE CARE UNIT PATIENTS IN A TERTIARY CARE HOSPITAL

Dissertation submitted to

THE TAMILNADU DR.M.G.R.MEDICAL UNIVERSITY

in partial fulfillment of the regulations for the

award of the degree of

M.D. (MICROBIOLOGY)

MADRAS MEDICAL COLLEGE,

THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY

CHENNAI – TAMILNADU

APRIL 2016

CERTIFICATE

This is to certify that this dissertation titled **"A STUDY ON BIOFILM FORMATION IN ORGANISMS CAUSING CENTRAL VENOUS CATHETER RELATED BLOOD STREAM INFECTION IN INTENSIVE CARE UNIT PATIENTS IN A TERTIARY CARE HOSPITAL''** is a bonafide record of work done by **DR.R.KRITHIGA,** during the period of her Post graduate study from 2012 to 2016 under guidance and supervision in the Institute of Microbiology, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai-600003, in partial fulfillment of the requirement for **M.D. MICROBIOLOGY** degree Examination of The Tamilnadu Dr. M.G.R. Medical University to be held in April 2016.

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I declare that the dissertation entitled **"A STUDY ON BIOFILM FORMATION IN ORGANISMS CAUSING CENTRAL VENOUS CATHETER RELATED BLOOD STREAM INFECTION IN INTENSIVE CARE UNIT PATIENTS IN A TERTIARY CARE HOSPITAL"** submitted by me for the degree of M.D. is the record work carried out by me during the period of **Oct 2014 to Aug 2015** under the guidance of Professor **DR.U.UMADEVI M.D.,** Professor of Microbiology ,Institute of Microbiology, Madras Medical College, Chennai. This dissertation is submitted to the Tamil Nadu Dr.M.G.R. Medical University, Chennai, in partial fulfillment of the University regulations for the award of degree of M.D., Microbiology (Branch IV) examinations to be held in April 2016.

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ACKNOWLEDGEMENT

I humbly submit this work to the Almighty who has given the health and ability to pass through all the difficulties in the compilation and proclamation of this blue print.

I wish to express my sincere thanks to our Dean, **Dr.R.Vimala, M.D.,** for permitting me to use the resources of this institution for my study.

I owe special thanks to **Prof. Dr.Mangala Adisesh, M.D.,** Director I/C, and Prof. **Dr.S.Vasanthi, M.D.,** Institute of Microbiology for their constant support and valuable suggestions.I feel fortunate and indebted to be under the guidance of **Prof.Dr.U.Uma Devi, MD.,** Professor, Institute of Microbiology, for her valuable advice, guidance in preparing and compilation of my work. She is a source of inspiration in my endeavours.

I express my gratitude to our former Director, **Prof. Dr.G.Jayalakshmi, M.D., DTCD** and my former guide **Dr.K.Muthulakshmi, M.D.** andformer Professor **Dr.T.Sheila Doris, M.D.**for their guidance and support.

I would like to thank my Professors **Dr.S.Thasneem Banu, M.D., Dr. R.Vanaja, M.D.,** for their valuable assistance in my study.

I extend my whole hearted gratitude and special thanks to my Assistant Professor **Dr.K.Usha Krishnan M.D.** for her valuable guidance, encouragement and constant support in my study.

I also express my thanks to our Assistant professors **Dr.R.Deepa, M.D., Dr.N.Rathnapriya,M.D., Dr.C.S.Sripriya,M.D., Dr.David Agatha M.D.**, **Dr.N.LakshmipriyaM.D.,DCH., Dr.K.G.Venkatesh M.D,Dr.B.Natesan M.D.,DLO** for their immense support in my study.

I hereby express my gratitude to all the technical staff for their help throughout my study.

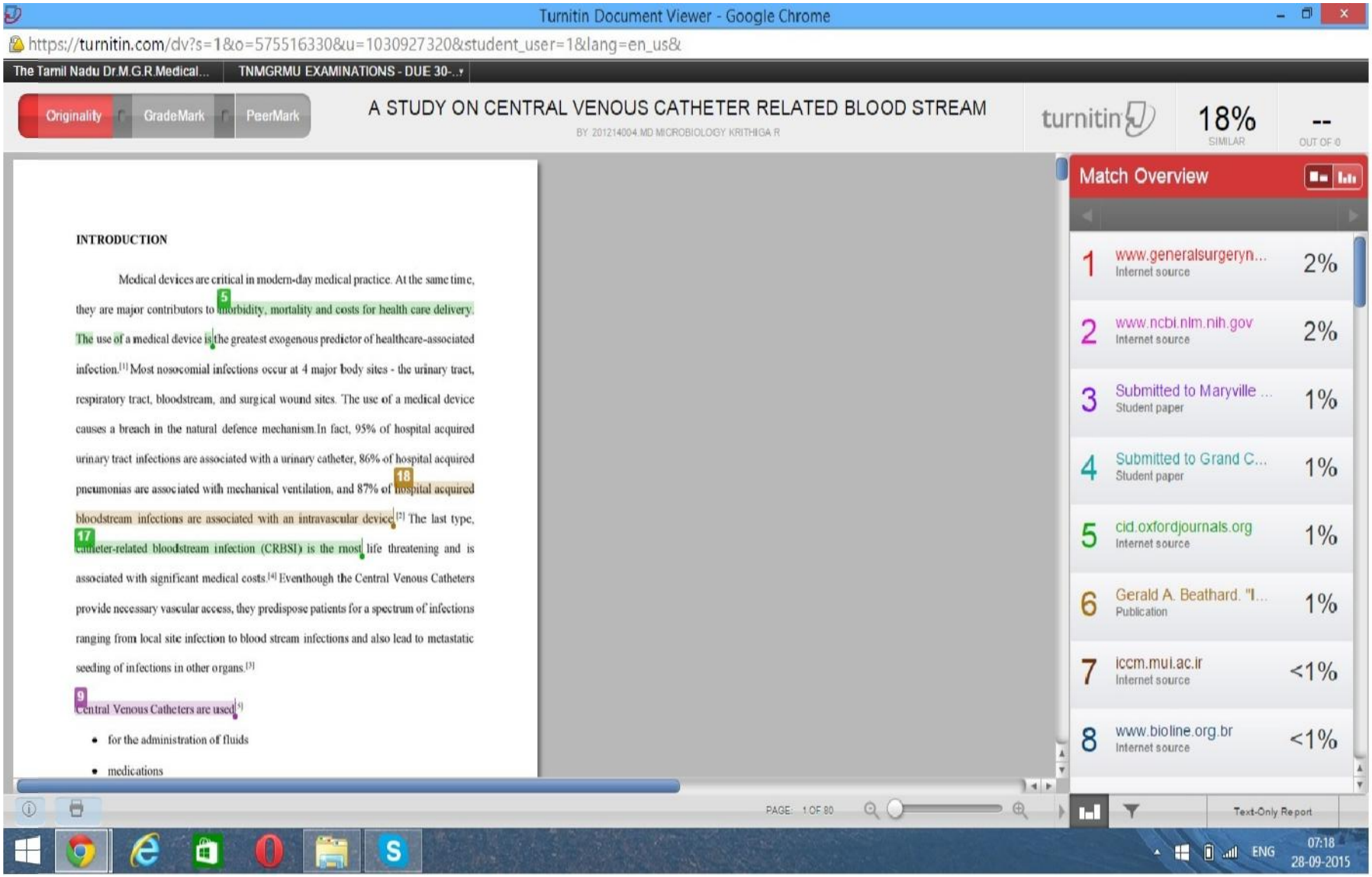
I would like to thank my department colleagues and friends for their constant support and co-operation.

I would like to thank the Institutional Ethics Committee for approving my study.

I would earnestly thank my parents for their constant encouragements.

I would like to thank my in-laws, my husband **Dr.B.S.Venkatesh M.S.** and my loving daughter who have sacrificed their family time and let me concentrate on my study. I thank them for being solid pillars of everlasting support and encouragement to me.

Finally, I am extremely thankful to my patients who consented and participated in my study.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INTRODUCTION
Medical devices are critical in modern-day medical practice. At the same time, they are major contributors to morbidity, mortality and costs for health care delivery. The use of a medical device is the greatest exogenous predictor of healthcare-associated infection.[1] Most nosocomial infections occur at 4 major body sites - the urinary tract, respiratory tract, bloodstream, and surgical wound sites. The use of a medical device causes a breach in the natural defence mechanism.In fact, 95% of hospital acquired urinary tract infections are associated with a urinary catheter, 86% of hospital acquired pneumonias are associated with mechanical ventilation, and 87% of hospital acquired bloodstream infections are associated with an intravascular device.[2] The last type, catheter-related bloodstream infection (CRBSI) is the most life threatening and is associated with significant medical costs.[4] Eventhough the Central Venous Catheters provide necessary vascular access, they predispose patients for a spectrum of infections ranging from local site infection to blood stream infections and also lead to metastatic seeding of infections in other organs.[3]
Central Venous Catheters are used.[5]
for the administration of fluids
medications
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A STUDY ON BIOFILM FORMATION IN ORGANISMS CAUSING CENTRAL VENOUS CATHETER RELATED BLOOD STREAM INFECTION IN INTENSIVE CARE UNIT PATIENTS IN A TERTIARY CARE HOSPITAL

ABSTRACT

BACK GROUND AND OBJECTIVES:

The aim of the study was to detect the presence of central venous catheter related blood stream infections caused by various microorganisms, their ability to form biofilms and their susceptibility patterns to various antimicrobial agents.

METHODOLOGY:

A total of 105 patients with clinical symptoms of CRBSI were included from Medical, Surgical and Trauma intensive care units. Catheter pull through blood samples accompanied with peripheral blood samples and CVC tip were processed at the Institute of Microbiology, Madras Medical College.

Isolates were identified based on standard microbiological methods and antimicrobial susceptibility testing was done. Biofilm formation was tested by three methods- tube method, microtitre plate method and Congo red Agar method.

RESULT:

Out of 105 patients, 16 showed significant growth of Laboratory Confirmed BloodStream infection. The infection rate of CRBSI was 14.2 per 1000 catheter days, incidence 15.23% and mortality rate 12.5%. In this study, 16 isolates were obtained for CRBSI in which Gram negative organisms accounted for 63% , Gram positive organisms accounted for 31% and Candida

species accounted for 6%. Of the Gram negative organisms, *Pseudomonas aeruginosa* was the commonest 25% followed by *Klebsiella pneumonia*e 19%, *Acinetobacter baumanii* 13%, *Klebsiella oxytoca* 6% and *Proteus vulgaris* 6%. Of the Gram positive organisms, *Staphylococcus aureus* was the commonest 19% followed by *Staphylococcus epidermidis* 6% .*Candida albicans* contributed to 6% of CRBSI.All the gram negative isolates were sensitive to Imipenem and all the Gram positive isolates obtained were sensitive to Vancomycin. In this study, among the gram negative organisms, 73% were biofilm producers and among Gram positive organisms, 50% were biofilm producers.100% of the Enterobacteriaceae isolates were ESBL producers.66% of the isolates were *Methicillin resistant Staphylococcus aureus*. Among the ESBL producers,60% were biofilm producers. Among the MRSA, 100% were biofilm producers.

CONCLUSION:

CRBSI are on the rise due to increased interventions, and are caused by common nosocomial pathogens which are still sensitive to reserve drugs and to very few routine drugs. Such infections can be controlled and prevented with stringent catheter care techniques and active surveillance measures.

KEY WORDS:

Central venous catheter, ICU, CRBSI (catheter related blood stream Infections), biofilm.

INTRODUCTION

Medical devices are critical in modern-day medical practice. At the same time, they are major contributors to morbidity, mortality and costs for health care delivery. The use of a medical device is the greatest exogenous predictor of healthcare-associated infection.[1]Most nosocomial infections occur at 4 major body sites - the urinary tract, respiratory tract, bloodstream, and surgical wound sites.The use of a medical device causes a breach in the natural defence mechanism. In fact, 95% of hospital acquired urinary tract infections are associated with a urinary catheter, 86% of hospital acquired pneumonias are associated with mechanical ventilation, and 87% of hospital acquired bloodstream infections are associated with an intravascular device.[2]The last type, catheter-related bloodstream infection (CRBSI) is the most life threatening and is associated with significant medical costs.[4] Eventhough the Central Venous Catheters provide necessary vascular access, they predispose patients for a spectrum of infections ranging from local site infection to blood stream infections and also lead to metastatic seeding of infections in other organs.[3]

Central Venous Catheters are used [5]

- for the administration of fluids
- medications
- parenteral nutrition

- blood products
- to monitor hemodynamic status
- to provide hemodialysis

Microorganisms introduced into the CVC can be [6]

- from the skin of the patient at the catheter insertion site
- from a contaminated catheter hub(health care worker hands)
- from hematogenous seeding of the device
- from infusion of contaminated infusate

Micro-organisms commonly attach to the medical devices and form biofilms that lead to colonization and sometimes infection. Biofilms are sessile microbial communities in which the organisms produce an extracellular polymeric substance (EPS) matrix.[10]About 65% of Hospital-acquired infections are caused by biofilm formation.[8]The process of biofilm formation is complex and in the case of central venous catheters, depends on multiple factors, such as the

- characteristics of the catheter material
- presence of a conditioning film
- hydrodynamics

- physical and chemical properties of the liquid in contact with the catheter surface

- properties of the microbial cells [7].

It has been reported that biofilms may form within 3 days after catheter insertion .[10] Biofilm formation is more predominant on the external surface of catheters in place for 10 days; however, with increasing catheter duration (>30 days), biofilm formation in the catheter lumen tends to predominate [7].

Biofilm organisms may elicit disease processes by

- detachment of individual cells or aggregates of cells from the device surface

- by production of endotoxins or other pyrogenic substances

- biofilms may provide a niche for the development of antimicrobial-resistant organisms by means of failure of antibiotic penetration, slow growing state in the biofilms causing reduced susceptibility and by means of different gene expressions in the planktonic and sessile counterparts.It has also been suggested that the negatively charged exopolysaccharide is very effective in protecting bacterial cells from cationic antibiotics by restricting their permeation.[8]

Gram-negative bacteria, Gram-positive bacteria and yeasts can form biofilms . The most common biofilm-forming bacteria include *Enterococcus*

faecalis, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus viridians*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Pseudomonas aeruginosa.* [9] Biofilms may be composed of a single species or multiple species, depending on the device and its duration of use in the patient. Thus the biofilm matrix may act as a filter, entrapping minerals or host-produced serum components and becomes tenacious.[10]

Catheter-related infections will continue to pose a serious threat unless prevention strategies, diagnostic techniques, and treatment modalities are implemented to address the pathogenic mechanisms of CRBSI and the microbiology of biofilms associated with vascular access devices.[11]

Hence keeping this view in mind, the present study is designed to detect the presence of central venous catheter associated blood stream infections caused by various bacteria, their ability to form biofilms and their susceptibility patterns to various antimicrobial agents in order to provide effective antibiotic strategy to reduce the incidence of hospital acquired blood stream infections.

AIMS & OBJECTIVES

AIMS AND OBJECTIVES

- To isolate and identify the bacterial and fungal organisms causing central venous catheter related blood stream infection.
- To analyse the biofilm forming potential of the organisms isolated.
- To study about antimicrobial susceptibility pattern of the isolates and to correlate the antimicrobial resistance with the biofilm formation

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Definitions

Healthcare-associated infection(HAI)[1] – An infection acquired in the hospital by a patient who was admitted for a reason other than the infection. An infection occurring in a patient in a hospital or other health care facility in whom the infection was neither present nor incubating at the time of admission. This includes infections acquired in the hospital but appearing after discharge and also occupational infections among staff of the facility.

Blood Stream Infection(BSI)[8] – two major categories of Blood stream infections are

Intravascular – those that originate within the cardiovascular system

Extravascular – those that result from bacteria entering the blood circulation through the lymphatic system from another site of infection

Catheter Related Blood Stream Infection (CRBSI)[13]– **C**atheterrelated infections are an intravascular form of Blood stream infections. CRBSIs contribute about 64% of nosocomial BSIs according to CDC's NNIS system (National Nosocomial Infection Surveillance System) and is attributed mainly to intravascular catheters particularly Central Venous Catheters for which the infection rate is expressed as number of CRBSIs per 1000 catheter days.

Patients having the following risk factors are more prone for HAI[12]

1) Age > 70 years
2) Shock
3) Major trauma
4) Acute Renal Failure
5) Coma
6) Prior antibiotics
7) Mechanical ventilation
8) Immunosuppressive drugs like steroids, chemotherapy.
9) Indwelling catheters
10) Prolonged ICU stay (> 3 days)

Epidemiology

Health care- associated infections(HAI) are an important cause of morbidity and mortality and place a significant burden on the health care system of which Central venous catheter related blood stream infections (CRBSI) account for 11% with an estimated mortality rate of 12 to 25%[15] and increased hospital cost[16]. In the United states,15 million CVC days occur in intensive care units each year.[14]Majority of CRBSIs are associated with

CVCs and in prospective studies, the relative risk for CRBSI is up to 64 times greater with CVCs than with peripheral venous catheters. [16]An estimate of 30,100 central line associated blood stream infections occur in U.S hospitals each year.[4]

Central Venous Catheterization.[4]

Central line is an intravascular catheter that terminates at or close to the heart or in one of the great vessels like Aorta, pulmonary artery, superior vena cava, inferior vena cava, Brachiocephalic veins, Internal jugular veins, Subclavian veins, External iliac veins, Common iliac veins, Femoral veins and umbilical artery / vein in case of neonates.

Types of Central line[2,4]

Temporary line – non tunnelled, non implanted catheter.

Permanent line – tunnelled catheters including dialysis catheters, Implanted catheters including ports.

Central venous catheters are now widely used in intensive care units. Like any medical procedure, CVC has specific indications and should be reserved for patients who potentially benefit from it.

Indications for Central Venous Catheterization[2]

1) Pulmonary artery catheterization
2) Total parenteral nutrition
3) Acute hemodialysis , plasmapheresis
4) Cardiopulmonary arrest
5) Emergency transvenous pacemaker
6) Hypovolemia, inability to perform peripheral iv
7) Preoperative preparation
8) General purpose venous access, vasoactive agents, caustic medications, radiologic procedures
9) Central venous oxygen saturation monitoring
10) Fluid management of ARDS (CVP monitoring).

Such central venous catheterization can be met with complications such as infection, pneumothorax, hemothorax, hematoma, thrombosis, arrhythmia and arterial puncture.[17]Of all the complications, Central venous catheter related blood stream infection(CRBSI) stands out to be the dreaded Healthcare-Associated Infection(HAI) for a patient admitted in an Intensive care unit with

high morbidity and mortality. Almost 80-90% of Blood stream infections(BSI) arising from vascular access are caused by CVCs.[3]

Factors influencing the risk of acquiring CRBSI[6,16,18]

1) Catheter characteristics- material, number of lumens, size, coating/ impregnation, frequency of catheter manipulations.

2) Reason for catheterization.

3) Catheters inserted in emergency situations.

4) Inexperienced person inserting the line, Improper site preparation, anatomical insertion site ,method of catheter insertion, purpose of insertion and duration of insertion

5) Standard of daily line care.

   The administration of parenteral nutrition through intravascular catheters , poor personal hygiene, occlusive transparent dressing, moisture around the exit site, Staphylococcus nasal colonization and contiguous infections support the role of bacterial colonization in the pathogenesis of CRBSI.

6) Patients admitted to intensive care unit are at higher risk than patients admitted in other wards and outpatients.

7) Colonization of patients with hospital acquired organisms.

Source of infection for CRBSI[10]

Microorganisms may originate from the skin of patients or health care workers ,tap water to which entry ports are exposed or other sources in the environment. The density of skin flora at the catheter insertion site is a major risk factor for CRBSI. Normally counts of 1000-10,000 cfu/cm^2 is present in jugular and subclavian catheter sites whereas 10 cfu/cm^2 at antecubital space. 80% of resident microorganisms inhabit the upper 5 layers of stratum corneum and 20% survive in biofilms within the epidermis, sebaceous glands and hair follicles.

Microbiology

Inthe past 2 decades, the antimicrobial resistant organisms such as methicillin resistant *Staphylococcus aureus*, multidrug resistant gram negative bacilli and fluconazole resistant Candida species is on the rise. [15]

The most common aetiological organisms for nosocomial CRBSI[9]are

Bacteria- *Staphylococcus aureus, Coagulase negative Staphylococcus,Pseudomonas aeruginosa, Acinetobacterbaumanii, Klebsiellapneumoniae ,Citrobacterfreundii, Enterococci and Escherichia coli.*

Fungi- mainly *Candida* species.

A prospective study using data from SCOPE(Surveillance and Control of Pathogens of Epidemiological importance) which included 24,179 cases of

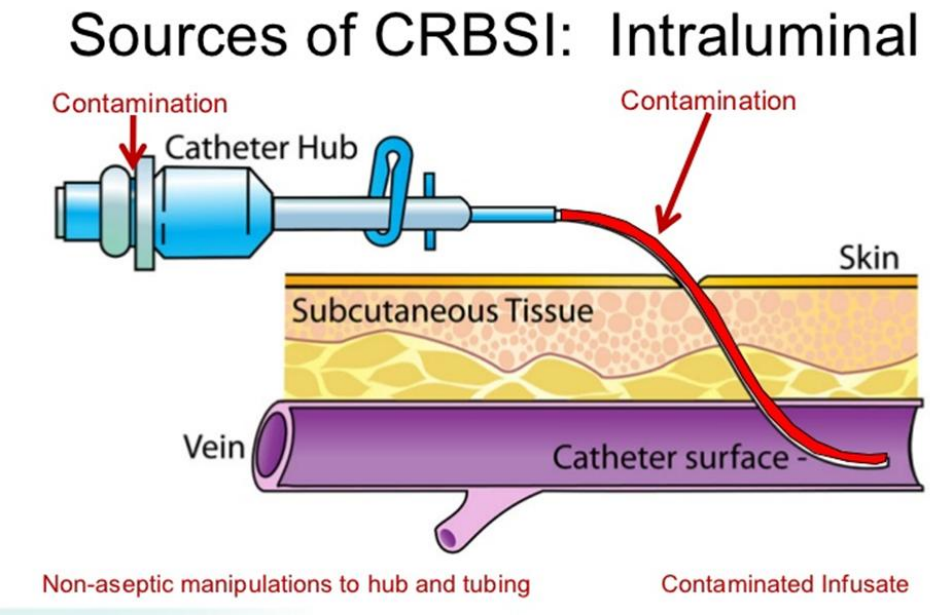
CRBSIs from a 7 year period at 49 hospitals found that the rates of MRSA isolates increased from 22% in 1995 to 57% in 2001($p< 0.001$) and rates of Ceftazidime resistant *Pseudomonas aeruginosa* isolates increased from 12% in 1995 to 29% in 2001($p<0.001$) and 60% of isolates contained Vancomycin resistant *Enterococcus faecium*. [19]

Pathophysiology of CRBSI

The pathogenesis of CRBSIs can be due to colonisation of catheter(from skin flora – extraluminally or from hematogenous seeding - intraluminally), due to contamination of the catheter hub or due to infusion of the contaminated infusate (this causes an epidemic which is almost rare) .[15]For short term CVCs(<10 days), the most common mode of colonization is along the external surface while for long term CVCs(>10 days), endoluminal spread from the hub appears to be the primary mechanism of infection.[16]

Direct contact of the microorganism with the catheter surface is required for attachment and subsequent colonization which happens by means of biofilm formation.[10] When the catheter is introduced into the venous system, the circulating plasma proteins collide and bind with the biomaterial which further activates the coagulation cascade and complement system attracting platelets and polymorphs. All the above process forms a conditioning layer that serves as a scaffold for the developing biofilm by providing receptor binding sites for newly arrived bacteria.[7]

Thus biofilm formation is the pathogenesis behind all device associated hospital acquired infections.[21]



About the Biofilm

Historical perspective

The first recorded observation concerning biofilm was probably given by Henrici in 1933 who observed that water bacteria are not free floating but grow upon submerged surfaces.[20] Nearly 40 years ago, Dr.R.J. Gibbons made the first report of his observations of polysaccharide glycocalyx formation on teeth by *Streptococcus mutans.*[21]

Present perspective

More recent direct microscopic observations and direct quantitative recovery techniques demonstrate that more than 99.9% of bacteria grow as

aggregated ‘sessile’ communities attached to surfaces rather than as ‘planktonic’ or free floating cells in liquid.[10]Biofilms are formed both on living tissue as well as nonliving inert material and are responsible for 65% of infections treated in the developing world.[9]

Definition of Biofilm[7]

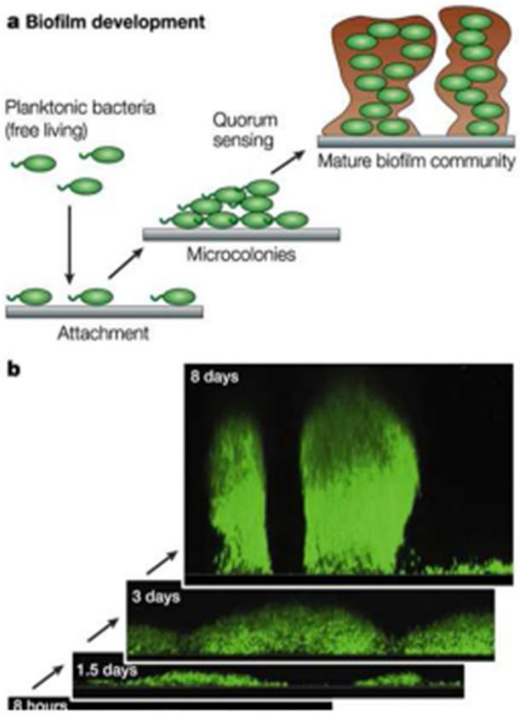
A biofilm is a primitive developmental biological system in which spatial organization of the cells within the matrix optimizes the use of available nutritional resources. An immobilized enzyme system is formed in which the milieu and enzyme activities are constantly changing and evolving to an appropriate steady state. This steady state can be radically altered by applying physical factors such as high shear force.

Factors influencing biofilm formation[21]

The potential causes behind formation of biofilms by bacteria during infection are

1) Protection from harmful conditions in the host(defense)
2) Sequestration to nutrition rich area(colonization)
3) Utilization of cooperative benefits(community)
4) Biofilms normally grow as biofilms and planktonic cultures are an in vitro artefact(biofilms as a default mode of growth) .

Steps in biofilm formation



Steps in biofilm formation[7,8,9,21]

1) Microbial attachment – microbes get attached to the conditioning layer.

2) Adhesion and microcolony formation – few minutes after microbial attachment, phenotypic changes occur in them and also upregulation of genes take place resulting in accumulation of proteins and polysaccharides which firmly adhere cells to the substratum. The cells continue to divide and the daughter cells thus formed become embedded in exopolymer saccharides(EPS) moving in upward and outward direction forming microcolonies. Thus the composition of microcolonies are 10% to 25% cells and 75% to 90% EPS.

3) Dispersion and Dissemination of Biofilm cells – dispersal is accompanied by shedding, detachment or shearing but they leave behind an adherent layer of cells on the surface to regenerate the biofilm. The number of organisms on the catheter tip is related to occurrence of bloodstream infection in the patient supporting the concept of a critical level of biofilm development above which substantial cell detachment and embolism occur.

Molecular mechanisms behind biofilm formation

The development and structural integrity of the biofilm depends on Quorum sensing(QS). QS is the ability to use extracellular molecules called pheromones to allow enhanced communication among bacteria. Pheromones are different for gram positive and gram negative bacteria. For gram positive organisms, the pheromones are oligopeptides or proteins whereas for gram negative bacteria, the pheromones are low molecular weight homoserine molecules such as N- acyl homoserine lactone. [7]

Biofilm recalcitrance to antimicrobials

The hallmark of biofilm is the innate resistance to antimicrobials and host immune responses due to the following factors

1) Restricted penetration - The negatively charged EPS restricts the positively charged antibiotics into the depths by binding to them and also restrict the passage of complement molecules.[10]

2) Nutrient limitation – limited nutrients and oxygen in the inners layers make the cells metabolically inactive and slow growing when compared to the active planktonic cells on the outer layers.[23]

3) Adaptive responses – due to fluctuations in temperature, pH, osmolarity and nutrient availability there occurs genetic alterations with expression of multiple stress response genes [21]

4) Genetic transfer – occurs by means of horizontal exchange of resistant plasmids between the biofilm cells[23]

5) Presence of persister cells - about 0.1% to 10% of biofilm cells remain as persister cells which ensure the survival of biofilm even in the escalated concentrations of antimicrobial agents.[10]

Clinical Presentation

A patient on CVC who presents with fever or chills, unexplained hypotension with no other localising sign is suspected to have CRBSI.[24] Mild symptoms are malaise and nausea while severe symptoms are high fever with rigors, hypotension, vomiting and changes in mental status.[25]

Exit site infection is indicated by the presence of erythema, swelling, tenderness and purulent discharge around the catheter exit and the part of the tunnel external to the cuff.[16] Severe sepsis and metastatic infectious complications such as infective endocarditis, septic arthritis, osteomyelitis, spinal epidural ascess and septic emboli can prolong the course of CRBSI.[26]

Diagnostic criteria for CRBSI

CRBSI was defined{ as isolation of the same organism from semiquantitative (more than 15 cfu) or quantitative culture (more than 100cfu / ml) of a catheter tip and a peripheral blood culture[27] or more than 3-5 fold growth in catheter pull through blood when compared to peripheral blood[15] or more than 100 cfu/ml growth of catheter pull through quantitative culture [15]} with systemic inflammatory response syndrome, after exclusion of other infection sources.

Diagnostic criteria for colonization

Colonization of the catheter tip was defined{ as the finding of>15 cfu of bacteria in semiquantitative culture and>10^2cfu / ml in quantitative culture from the catheter tip in a patient without growth in the peripheral blood culture or growth in catheter pull through blood sample with no growth in peripheral blood culture or less than 100 cfu/ml growth of catheter pull through sample } without clinical symptoms of sepsis.[27]

Laboratory confirmed Blood stream infection criteria (LCBI).[4]

LCBI 1 –Patient has a recognized pathogen cultured from one or more blood cultures and organism cultured from blood is not related to an infection at another site.

LCBI 2 – Patient has atleast one of the following signs or symptoms : fever (>38°C),chills or hypotension and positive laboratory results are related to an

infection at any other site and the same common commensal(i.e. *diphtheroids, Bacillus sp, Propionibacteriumsp, Coagulase negative Staphylococcus, viridans group Streptococci, Aerococcus and Micrococcus*) is cultured from two or more blood cultures drawn on separate occasions.

LCBI 3 – Patient <= 1 year of age has atleast one of the following signs or symptoms: fever(>38°C), hypothermia (36°C core),apnoea or bradycardia and the same common commensal(i.e. *diphtheroids, Bacillus sp, Propionibacteriumsp, Coagulase negative Staphylococcus, viridans group Streptococci, Aerococcus and Micrococcus*) is cultured from two or more blood cultures drawn on separate occasions.

Catheter associated blood stream infections(CABSI) –

A laboratory-confirmed bloodstream infection (LCBI) where central line (CL) or umbilical catheter (UC) was in place for >2 calendar days on the date of event, with day of device placement being Day 1, and a CL or UC was in place on the date of event or the day before. If a CL or UC was in place for >2 calendar days and then removed, the LCBI criteria must be fully met on the day of discontinuation or the next day. If the patient is admitted or transferred into a facility with a central line in place (e.g., tunnelled or implanted central line), and that is the patient's only central line, day of first access as an inpatient is considered Day1. "Access" is defined as line placement, infusion or withdrawal through the line.[4]

Methods for diagnosis of CRBSI[15]

Methods not requiring CVC removal

Diagnostic method	Description	Criteria for positivity	Sensitivity %	Specificity %
Qualitative blood culture through device	One or more blood cultures drawn through CVC	Any growth	87	83
Quantitative blood culture through device	Blood culture drawn through CVC, processed by pour plates or lysis centrifugation	>= 100 cfu / ml	77	90
Paired Quantitative blood cultures	Simultaneous cultures drawn through CVC and percutaneously	Both cultures positive with CVC culture yielding 5 fold higher or more than peripherally drawn culture	87	98
Differential time to positivity	Simultaneous blood cultures drawn through CVC and percutaneously and monitored continuously	Both cultures positive with CVC positive >= 2 hours earlier than peripherally drawn culture	85	81

Methods requiring CVC removal

Diagnostic method	Description	Criteria for positivity	Sensitivity %	Specificity %
Qualitative catheter segment culture	Segment from removed CVC is immersed in broth media and incubated for 24-72 hours	Any growth	90	72
Semiquantitative catheter segment culture	A 5 cm segment from removed CVC is rolled 4 times across a blood agar plate and incubated	>= 15 cfu	85	82
Quantitative catheter segment culture	Segment from removed CVC is flushed or sonicated with broth , serially diluted and plated on blood agar	>=1000cfu	83	87
Microscopy of stained CVC: Gram stain and acridine orange staining	Direct visualisation of the microorganisms		84-100%	97-100%

Prevention of CRBSI

The Centre for Disease Control and Prevention(CDC) and Healthcare Infection Control Practices Advisory Committee(HICPAC) devised guidelines intending to provide Evidence based recommendations for preventing CRBSIs.

Education, Training and Staffing[28,29]

1) Educating the healthcare personnel regarding the indications for CVC use, proper procedures for insertion and maintenance of CVCs and appropriate infection control measures to prevent CRBSIs.
2) Limiting the staffs in ICUs to decrease CRBSI.
3) Designate only trained personnel and periodically assess their knowledge.

Surveillance of Catheter related infection[28]

1) Inspection and palpation of the catheter sites through intact dressing.
2) Record the operator, date and time of catheter insertion and removal and dressing changes on a standardised form.

Selection of Catheters and sites[17]

1) CVCs are recommended only if the benefits outweigh the risks.
2) Subclavian site is better than jugular or femoral site.
3) USG guided CVC insertion can be done to reduce the number of cannulation.
4) CVC with minimum number of ports or lumens should be used.
5) Remove CVC if it is no longer needed.
6) When aseptic techniques have not been followed in emergent situations, replace the CVC within 48 hours.

Type of Catheter material [30]

Polytetrafluoroethylene (Teflon) or polyurethane catheters have been associated with fewer infectious complications than catheters made of polyvinyl chloride or polyethylene.

Hand hygiene and Aseptic technique[14,28,29]

1) Hand washing with conventional soap or rubbing with Alcohol based handrub should be done before and after palpating catheter insertion site, before and after inserting , replacing or dressing a CVC.
2) Aseptic technique for the insertion and care of CVC is a must which means wearing sterile gloves.

Maximal Sterile Barrier Precautions [29]

A cap, mask, sterile gown, sterile gloves and a sterile full drape for insertion of CVC is mandatory.

Skin Preparation[29,30]

1) Prepare clean skin with a 0.5% Chlorhexidine preparation with alcohol before CVC insertion. If there is a contraindication for chlorhexidine, tincture of iodine, an iodophor or 70% alcohol can be used.
2) Antiseptics should be allowed to dry before insertion of CVC.

Catheter Site Dressing Regimens[14]

1) Use sterile gauze or sterile transparent semipermeable membrane dressing to cover the catheter site.
2) If the site is bleeding or oozing, use gauze dressing until it is resolved.
3) Replace dressing if it becomes damp, loosened or soiled.
4) Avoid antibiotic creams as it promotes fungal infection.
5) Avoid showering over the catheter site.
6) Replace short term CVC dressings every 2 days for gauze dressings and every 7 days for transparent dressings.
7) Encourage patients to report any changes in the catheter site or any discomfort to the healthcare provider.

Patient cleansing[14]

Use a 2% chlorhexidine wash for daily skin cleansing.

Catheter Securement Devices[14,28]

Using sutureless securement device avoiding disruption around catheter entry site decreases infection.

Antimicrobial / Antiseptic impregnated Catheters and Cuffs[14,28,30]

Even after successful implementation of Comprehensive strategy which includes educating staffs, following maximal sterile barrier precautions and >0.5% chlorhexidine preparation with alcohol for skin antisepsis, the

CRBSI rate is not decreasing then use chlorhexidine / silver sulphadiazine or minocycline / rifampin or platinum / silver impregnated CVCs.

Systemic Antibiotic Prophylaxis[28]

Do not administer systemic antimicrobial prophylaxis before insertion or during use of CVC to prevent colonization or CRBSI.

Anticoagulants[14]

Do not routinely use anticoagulant therapy to reduce the risk of catheter related infection.

Replacement of CVCs[14,28]

1) Select the catheter, insertion technique and insertion site with the lowset risk of complications(infectious and non-infectious) for the anticipated type and duration of intravenous therapy.
2) Do not routinely replace the catheters for the purpose of reducing infection.
3) Clinically a patient should be judged for infection elsewhere or for a non-infectious cause of fever before catheter removal is done.
4) Use a guidewire exchange to replace malfunctioning non tunnelled catheter only if there is no evidence of infection .
5) Replace any short term CVC if purulence is observed at the insertion site which indicates infection.

6) Replace all CVCs if the patient is haemodynamically unstable and CRBSI is suspected.

Replacement of Administration sets[14]

1) In patients receiving blood, blood products or fat emulsions ,tubings should be replaced within 24 hours. Otherwise 96 hour interval can be given before replacement.
2) Replace tubing used to administer propofol solutions every 6 or 12 hours.

Hangtime for parenteral fluids[28]

1) Complete infusions of lipid containing fluids within 24 hours of hanging the fluid.
2) Complete infusions of blood or other blood products within 4 hours of hanging the blood.

Preparation and Quality control of intravenous admixtures[14,17,28]

1) Admix all routine parenteral fluids in a laminar flow hood using aseptic techniques.
2) Do not use infusate if the container has leaks, cracks, turbidity, particulate matter or if the expiry date has passed.
3) Use single dose vials for parenteral additives or medications.
4) If multidose vials are used, refrigerate them and clean the access diaphragm with 70% alcohol
5) Do not use inline filters routinely for infection control purposes.

Surveillance[14]

1) Conduct surveillance in ICUs and other patient populations to determine CRBSI rates, monitor trends in those rates and assist in identifying lapses in infection control practices.
2) Express ICU data as the number of catheter associated blood stream infections per 1000 catheter days for both adults and children.

Peter Pronovost et al conducted an intervention study recommending procedures such as handwashing, full barrier precautions during the insertion of catheter , cleaning the skin with chlorhexidine , avoiding the femoral site if possible and removing unnecessary catheters. The median CRBSI rate decreased from 2.7 per 1000 catheter days to 0 and the mean rate decreased from 7.7 to 1.4 per 1000 catheter days. The incidence rate decreased from 0.62 to 0.34. [13]

Considering environmental factors[29]

1) High quality cleaning and disinfection of all patient care areas is very important
2) The ICU unit should be situated close to the operation theatre and emergency department and away from wards
3) Suitable and safe air quality should be maintained
4) There should be separate areas for clean storage and waste disposal

Performance indicators for prevention of Catheter Associated Blood Stream Infection. [28]

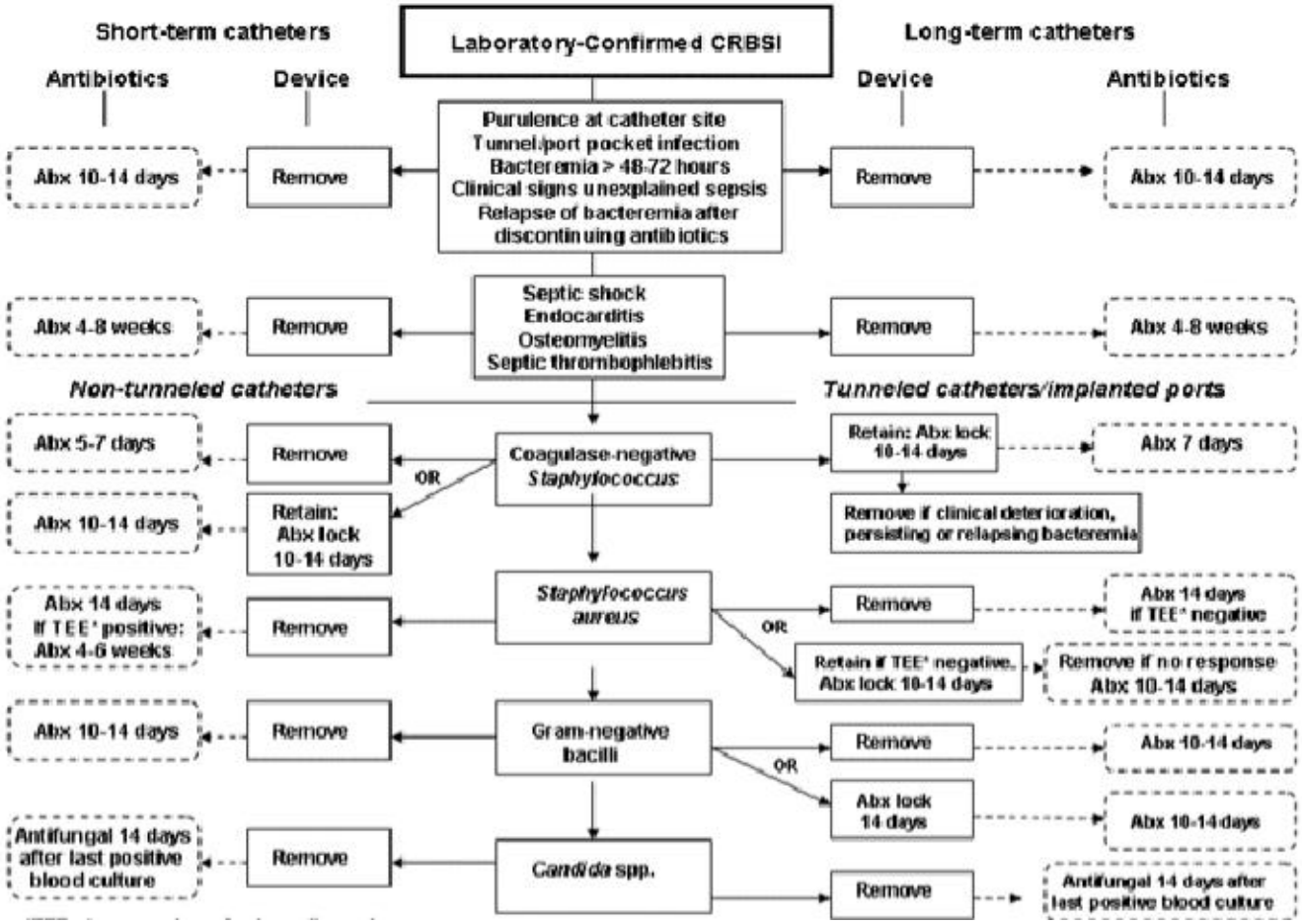
1) Implementation of educational programmes that include didactic and interactive components for those who insert and maintain catheters.
2) Use of maximum sterile barrier precautions during catheter placement.
3) Use of chlorhexidine for skin antisepsis.
4) Rates of catheter discontinuation when the catheter is no longer essential for medical management.

Management of CRBSI

Depends mainly on two strategies

1) Appropriate and timely administration of systemic antimicrobial treatment. [15]
2) Treatment of the catheter associated biofilm as the source of infection either by catheter removal or catheter salvage.[10]

Empirical broad spectrum antimicrobial therapy is initiated after the collection of appropriate samples, depending on the pathogen profile present in a given institution and the severity of the patients illness. [10]


Short-term catheters
Laboratory-Confirmed CRBSI
Long-term catheters
Antibiotics
Device
Device
Antibiotics
Purulence at catheter site
Tunnel/port pocket infection
Bacteremia > 48-72 hours
Clinical signs unexplained sepsis
Relapse of bacteremia after discontinuing antibiotics
Abx 10-14 days
Remove
Remove
Abx 10-14 days
Septic shock
Endocarditis
Osteomyelitis
Septic thrombophlebitis
Abx 4-8 weeks
Remove
Remove
Abx 4-8 weeks
Non-tunneled catheters
Tunneled catheters/implanted ports
Abx 5-7 days
Remove
Coagulase-negative Staphylococcus
Retain: Abx lock 10-14 days
Abx 7 days
OR
Abx 10-14 days
Retain: Abx lock 10-14 days
Remove if clinical deterioration, persisting or relapsing bacteremia
Abx 14 days if TEE* positive: Abx 4-6 weeks
Remove
Staphylococcus aureus
Remove
Abx 14 days if TEE* negative
OR
Retain if TEE* negative, Abx lock 10-14 days
Remove if no response Abx 10-14 days
Abx 10-14 days
Remove
Gram-negative bacilli
Remove
Abx 10-14 days
OR
Abx lock 14 days
Abx 10-14 days
Antifungal 14 days after last positive blood culture
Remove
Candida spp.
Remove
Antifungal 14 days after last positive blood culture

Antibiotic lock therapy

ALT involves installing a higher oncentration of an antibiotic to which the causative microbe is susceptible in the catheter lumen.[27]

Vancomycin at 5 mg/ml is more efficacious in eradicating Staphylococci embedded within biofilm.[31] and also for ampicillin resistant Enterococci other than Vancomycin resistant Enterococci.

Ceftazidime, gentamicin or ciprofloxacin can be used for the treatment of Gram negative organisms.

Berrington and Gould studied that bacteriostatic agents be used better for ALT.[32]

Atleast 1-5 ml of the ALT solution should be used to fill catheter lumen. [33]

It is suggested that the dwell time for Alt be better ≥12 hours for about 14 days. [22]

Ethanol Lock Therapy (ELT) [22]

Ethanol is an antiseptic agent which exhibits bactericidal and fungicidal activity against a wide range of organisms including Gram-negative and Gram-positive bacteria. It is readily available, inexpensive, and currently no resistance to microorganisms has been discovered. In contrast to antibiotic lock therapy, ethanol works by denaturisation therefore the effect does not

depend on microorganism resistance or sensitivity. For these reasons this therapy has sparked interest within the medical field to safely develop a standard process for its use in the prevention and treatment of CRBSI.

Eliminating Biofilms on medical devices [22]

The invitro testing models use a variety of antimicrobials and other chemical substances for testing.

Antimicrobials

Antimicrobials such as Rifampin, alone and along with glcopeptides, fluoroquinolones and macrolides have shown effect in reducing the EPS of biofilm biomass. Gentamicin has shown to reduce the Mininmum biofilm inhibitory concentration of ampicillin, Vancomycin and linezolid for Enterococcus. [34]

Chelating agents

Chelating agents for calcium , magnesium and iron such as EDTA(Ethylene Diamine Tetra Acetic acid) is found to have antimicrobial activities against a spectrum of organisms. EDTA in combination with antibiotics like minocycline, tigecycline and gentamicin can also be used.[35,36,37]

Sodium citrate has also shown effect against biofilm of most Gram positive organisms. A combination of 7% trisodium citrate, 0.05% methylene

blue,0.15% methyl paraben and 0.015% propyl paraben exhibit antibiofilm activity of Staphylococcus aureus.[38]

A combination of 4% trisodium citrate and 30% ethanol can also be used against biofilm of *S.aureus, S.epidermidis, P.aeruginosa and E.coli* for 72 hours in vitro. [39]

Ethanol

20% ethanol for 24 hours , 40% for 1hour, 60%- 80% for 1 min has shown a promising invitro activity against 24 hour biofilms of S.epidermidis, S.hominis and S.capitis. 25% ethanol along with minocycline(3mg/ml) and EDTA(30 mg/ml) resulted in complete eradication of S.aureus biofilms.[22]

Biofilm dispersant

Dispersants such as oxidizing biocides like chlorine, surfactants and enzymes such as cis 2- decanoic acid (an unsaturated fatty acid produced by P.aeruginosa) can induce dispersion of biofilm cells which along with bactericidal agents can prevent reattachment. [22]

Bacteriophage

Polysaccharide depolymerases produced by some phage strains can degade the biofilm EPS.[22]

N-acetylcysteine

A mucolytic which interferes with the exopolysaccharide formation in biofilms.[40]

GlmU enzyme inhibitor

N-ethyl maleimide and Protamine sulphate are the inhibitors against GlmUenzyme(N acetylglucosamine-1-phosphate uridyltransferase) , an enzyme required for peptidoglycan synthesis and lipopolysaccharide synthesis in Gram positive and Gram negative bacteria respectively.[40]

Silver nanoparticles[20]

Nanotechnology is useful for biofilm penetration and reducing biofilm formation. AgNPs hydrogel hybrid with different sizes of AgNPs can be effectively employed as antibacterial agents.

MATERIALS AND METHODS

MATERIALS & METHODS

Ethics consideration

Ethics committee clearance [EC RegNo.ECR/270/Inst./TN/2013] was obtained from the Institutional Ethics Committee, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai-3.

Study design **:** Cross sectional study

Study period **:** October 2014 to August 2015

Study setting:

The study was conducted in the Institute of Microbiology in association with the Intensive Medical, Surgical and Trauma care units, Madras Medical College &RGGGH . All patients satisfying the following inclusion criteria were recruited. Patients' clinical history was collected by a standard proforma.

Sample Size **:** 105

Inclusion criteria:

- ICU patients >18ys of age with indwelling central venous catheter who developed symptoms of blood stream infections after 48hrs of catheterization were included in the study.
- fever (temp ≥ 38°C) without any other known cause in patients with indwelling central venous catheters.

- Overt catheter site infection, i.e. any 2 of the following- erythema, tenderness and purulent exudate.

Exclusion criteria:

- Patients diagnosed with clinical syndromes such as pneumonia, urinary tract infection, cellulitis, septicaemia and infective endocarditis were excluded .
- Patients with Retroviral disease.
- Patients on immunosuppressive drugs.
- Patients in whom central venous catheterization was done elsewhere.

Samples were collected as per the following categories of patients based on the clinical status and indication.

<u>Category[1]-</u> Patients with suspected CRBSI with maintenance Central venous catheterization

1)Catheter blood sample

2)Peripheral blood sample

Peripheral blood samples were collected within 15 minutes of collection of central venous blood**.**

Category[2]-Patients with suspected CRBSI in whom maintenance catheterization is not indicated and central venous catheter can be removed

1) Catheter tip sample

2) Peripheral blood sample

Peripheral Blood sample should be collected within 15 minutes of catheter removal

Category[3]- For patients with difficult peripheral vein access

1) Catheter blood sample (For Quantitative culture through device)

SAMPLE COLLECTION:

Under strict aseptic precautions samples were collected from the patients and transported immediately to the laboratory appropriately for further process.

Method for Catheter tip collection

The skin was disinfected with 70% alcohol[42]or 2%chlorhexidine[43] before catheter removal. The catheter was held with the proximal end, removed aseptically without touching the skin and the distal 5 to 6 cm cut off with sterile scissors into a sterile tube which was sent to the laboratory within 30 minutes to avoid drying.[42]

Method for Catheter blood collection

- **For Qualitative culture**

The catheter hub was cleaned with 70% alcohol and allowed to evaporate after which 10 ml of blood was collected and added to Tryptic soy broth .

- **For Quantitative culture**

The catheter hub was cleaned with 70% alcohol and allowed to evaporate after which 1ml of blood was collected and heparinised(0.1ml of 50,000IU heparin).[44]

Method of Peripheral blood Collection

The venepuncture site was first cleaned with povidone iodine and then with 70% alcohol. A tourniquet was applied above the venepuncture site. With sterile aseptic precautions, about 10ml of blood was obtained and inoculated into a blood culture bottle containing 50 mlTryptic soy broth.[45]

SAMPLE PROCESSING:

1. **Catheter tip:**

Semiquantitative Maki's Roll plate method (Exoluminal method)

Using sterile forceps, the catheter tip was taken from the sterile tube and placed on the blood agar . The tip was then rolled back and forth about four times over the entire surface of agar.[42]

Quantitative Brun Buisson's tip flush method (Endoluminal method)

Sterile normal saline 1ml was dripped into the lumen of the tube inside a sterile tube and 0.1ml of the flushed material was evenly spread onto the blood agar plate.[47]

2. **Catheter blood:**

Qualitative culture

10 ml of blood which was collected and added to Tryptic soy broth was incubated at37°C . Subcultures onto Mac conkey agar and sheep Blood agar plates were done once on the first day and twice within 3 days. The broth was incubated and inspected for upto 5-7 days with a final subculture. [46]

Quantitative culture

1ml of blood which was collected and heparinised(0.1ml of 50,000IU heparin) was added to 20 ml of melted Tryptic soy agar after cooling it to 45-50°C, poured into sterile petri dishes, incubated at37°C overnight and colonies counted.[43]

3. Peripheral blood

10 ml of blood which was collected in 50 ml of Tryptic soy broth was incubated at37°C for 48 hours and subcultured onto Mac conkey Agar and Blood Agar plates, incubated at37°C . Subcultures onto Mac conkey agar and sheep Blood agar plates were done once on the first day and twice within 3 days. The broth was incubated and inspected for up to 5-7 days with a final subculture. [46]

IDENTIFICATION OF MICROORGANISMS:

The colonies grown were further identified using Gram's staining and Biochemical reactions.[48]

Gram staining:-

Smears were prepared from the growth, followed by gram staining. The control strains used were S. aureus (ATCC 25923) and E.coli (ATCC 25922).

According to Gram reaction, the organisms were subjected to biochemical reactions using appropriate quality controls. [48]

Biochemical reactions :

For identification of Gram positive cocci

1) Catalase test
2) Modified Oxidase test
3) Coagulase test
4) Urease test
5) Aminoacid decarboxylation test(Lysine and Ornithine)
6) Arginine dihydrolation test
6) Sugar fermentation test with glucose, lactose, sucrose, maltose, mannose, mannitol, xylose.
7) Bile esculin test
8) Differential discs-Novobiocin[5µg,furazolidone[100µg] ,Bacitracin [0.04 units/disk]

For identification of Gram negative bacilli

1) Hanging drop – to check for motility
2) Catalase test
3) Oxidase test

4) Nitrate reduction test

5) Indole production test

6) Methyl red test

7) VogesProskauer test

8) Citrate utilization test

9) Urease test

10) Triple sugar iron agar test

11) Sugar fermentation test Glucose, Lactose, Sucrose ,maltose and mannitol.

12) Amino acid decarboxylation tests

13) Hugh-Leifson's Oxidation Fermentation test

For identification of Candida species:[52]

1) Germtube test

2) Fermentation of 2% sugars

3) Chrome Agar

ANTIMICROBIAL SUSCEPTIBILITY TESTING

The antimicrobial sensitivity of aerobic bacterial isolates was performed on Mueller Hinton agar (MHA) plates by standardized Kirby Bauer disc diffusion technique as per the CLSI (Clinical Laboratory Standards Institute) guidelines M100-S24 [January 2014][50]. Antifungal susceptibility testing according to CLSI guidelines M44-A[2004]. [51]

ANTIBACTERIAL SUSCEPTIBILITY TESTING[49]

Three to five identical colonies were picked from an overnight grown primary agar plate with a sterile loop and were suspended in 0.5ml of sterile saline. The turbidity was matched with 0.5 McFarland turbidity standards. A fresh, sterile cotton tipped swab was dipped into this suspension and the excess of inoculum was removed by pressing it against the sides of the tube. The surface of Mueller Hinton agar plate was inoculated, by starting at the top and streaking back and forth from edge to edge. The plate was rotated approximately at 60° and swabbing repeated for three times. The antibiotic discs were placed on the plate, so that even contact was ensured using sterile forceps after 15 minutes of inoculation and incubated aerobically at 37°C . After 18-24 hours of incubation, the diameter of the clear zone around the disk was measured under transmitted light with measuring scale and results were interpreted as susceptible, intermediate or resistant as per the CLSI criteria. The quality control for antimicrobial susceptibility testing was done with

standard strains of E.coli (ATCC 25922), S. aureus (ATCC 25923) and P. aeruginosa (ATCC 27853).

The drugs(Himedia) used for Gram positive organisms were:- [50]

Antibiotic	Disc content	Gram positive cocci	Diameter of zone of inhibition in mm		
			Sensitive	Intermediate	Resistant
Penicillin	10 units	*Staphylococcus species*	≥ 29	-	≤ 28
Cefoxitin	30 µg	*Staphylococcus aureus*	≥ 22	-	≤ 21
		CoNS	≥ 25	-	≤ 24
Amikacin	30 µg	*Staphylococcus species*	≥ 17	15-16	≤ 14
Erythromycin	15 µg	*Staphylococcus species& Enterococcus species*	≥ 23	14-22	≤13
		S.pneumoniae	≥ 21	16-20	≤15
Ciprofloxacin	5 µg	*Staphylococcus species& Enterococcus species*	≥ 21	16-20	≤15
Trimethoprim-Sulfamethoxazole	1.25/ 23.75µg	*S.pneumoniae*	≥ 19	16-18	≤15
Tetracycline	30 µg	*Enterococcus species& Staphylococcus species*	≥ 19	15-18	≤14

The drugs (Himedia)used for Gram negative organisms were:- [50]

Antibiotic	Disc content	Gram negative bacilli	Diameter of zone of inhibition in mm		
			Sensitive	Intermediate	Resistant
Amikacin	30 µg	*Enterobacteriaceae &Pseudomonas aeruginosa*	≥ 17	15-16	≤ 14
Gentamicin	10 µg	*Enterobacteriaceae &Pseudomonas aeruginosa*	≥15	13-14	≤12
Cefotaxime	30µg	*Enterobacteriaceae*	≥26	23-25	≤22
Ceftazidime	30µg	*Pseudomonas aeruginosa*	≥18	15-17	≤14
Ciprofloxacin	5 µg	*Enterobacteriaceae &Pseudomonas aeruginosa*	≥ 21	16-20	≤15
Cotrimoxazole	1.25/ 23.75µg		≥16	11-15	≤10
Piperacillin-tazobactam	100µg/10 µg	*Pseudomonas aeruginosa*	≥21	15-20	≤14
Tetracycline	30 µg	*Enterobacteriaceae*	≥15	12-14	≤11
Imipenem	10µg	*Enterobacteriaceae* *P.aeruginosa* *Acinetobacter sp.*	≥23 ≥19 ≥16	20-22 16-18 14-15	≤19 ≤15 ≤13

ANTIFUNGAL SUSCEPTIBILITY TESTING[51]

This method was carried out following the M 44-A CLSI guidelines using fluconazole and voriconazole discs (Himedia).

Antifungal disc	Disc content	Diameter of zone of inhibition in mm		
		Sensitive	Susceptible Dose Dependent	Resistant
Fluconazole	25 µg	≥17	14-16	≤13
Voriconazole	1 µg	≥17	14-16	≤13

Inoculum preparation and application of discs:[52]

- With a sterile bacteriological loop, 3- 5 yeast colonies were taken from the culture grown on SDA and emulsified in 5ml of sterile saline.
- The yeast suspension was matched to a 0.5 McFarland standards.
- By using a sterile cotton swab, the suspension was streaked in three directions on to the surface of a Mueller Hinton Agar plate supplemented with 2% glucose and 0.5µg/ml methylene blue.

- By using sterile forceps,fluconazole and voriconazole discs were placed on the surface of the agar.

The plates were incubated at 37^{o}C. After 24 hours of incubation, the diameter of zone of inhibition was measured and interpreted as sensitive or resistant according to the CLSI guidelines. Quality control strain used-ATCC Candida albicans 90028.

Detection of methicillin resistance in *Staphylococcus aureus* and *Coagulase negative Staphylococci(CoNS)* isolates by cefoxitin disc diffusion test [50]:

All *Staphylococcus aureus* and *Coagulase negative Staphylococci(CoNS)* isolates were subjected to cefoxitin disc diffusion test. Cefoxitin is used as a surrogate for *mecA*-mediated oxacillin resistance. The bacterial suspension of test isolateswere matched to a 0.5 McFarland standards and lawn cultured on Mueller Hinton Agar plates separately.Cefoxitin(30µg) disc were placed on the surface of lawn culture of the isolates. The plates were incubated in ambient air at 35°Cfor 24 hours.Quality control strain used - *Staphylococcus aureus* ATCC 25923.

Interpretation as per CLSI guidelines:

Isolate	**Sensitive Zone of inhibition (mm)**	**Resistant Zone of inhibition (mm)**
Staphylococcus aureus and Staphylococcus lugdunensis	≥22mm	≤21mm
Coagulase negative Staphylococci except *Staphylococcus lugdunensis*	≥25mm	≤24mm

Screening Test for Extended -Spectrum β-Lactamases (ESBLs) in Enterobacteriaceae isolates [50]

All the *Enterobacteriaceae* isolates were subjected to initial screening test for Extended -Spectrum β-Lactamases by using Cefotaxime and Ceftazidime discs by disc diffusion as per CLSI guidelines. The use of more than one antimicrobial agent for screening improves the sensitivity of ESBL detection.

Procedure:

The bacterial suspension of test isolates were matched to a 0.5 McFarland standards and lawn cultured on Mueller Hinton Agar plates separately. Ceftazidime (30 µg) and Cefotaxime(30 µg) discs were placed on the surface of lawn culture of the isolates. The plates were incubated in ambient air at 37°Cfor 16-18 hours.

Interpretation:

Ceftazidime zone ≤22 mm ,Cefotaxime zone ≤27 mm may indicate ESBL production.

MINIMUM INHIBITORY CONCENTRATION BY EPSILOMETER TEST (E-TEST)[53]

All MRSA isolates were subjected to MIC estimation against

Vancomycin by using E-test method (HI-MEDIA).

The E-test strips contains antimicrobial agent with a continuous exponential gradient of antibiotics from 0.016µg to 256 µg immobilized on porous paper material and MIC values printed on both sides identically .

Procedure:

The strains were inoculated into tubes containing 2ml of peptone water. The suspension was sreaked onto the Mueller Hinton Agar with2% Nacl to give a lawn culture. E-test strips were placed on the inoculated plates. The plates were incubated at 37°C for 24 hours and reading was taken the next day. MIC of the drug was taken at the point where the ellipse intersects the MIC scale on the strip. Control strain *ATCC Staphylococcus aureus* 25923 were tested in parallel.

Interpretation: As per CLSI guidelines

MIC value	Interpretation
≤2 µg/ml	sensitive
4-8 µg/ml	intermediate
≥16 µg/ml	resistant

BIOFILM DETECTION METHODS

Tube method[54]

10 ml of Tryptic soy broth with 1% glucose was inoculated with loopful of microorganisms from overnight culture plates, then incubated for 24 hours at 37°C. The tubes were then decanted and washed with Phosphate buffer Saline (pH- 7.3). The dried tubes were stained with 0.1% crystal violet.Excess stain was removed with deionized water and dried in inverted position.

Positive control- *Pseudomonas aeruginosa ATCC 27853*

Negative control- *Escherichia coli ATCC 25922*

Interpretation:

Positive – Visible film lining the walls and bottom of the tube

Scoring: 0- absent, 1-weak, 2- moderate, 3- strong

Ring formation at the liquid interface is considered negative.

Congo red agar method[54]

The Congo red agar medium consists of Brain heart Infusion broth(37 g/ L), sucrose(50 g/L), agar no.1(10g/L) and Congo red stain(0.8 g/L). Congo red was prepared as concentrated aqueous solution and autoclaved at121°C for 15 mins separately and then added to the agar cooled to 55°C. Plates were inoculated and incubated aerobically for 24 to 48 hours at 37°C.

Positive control- *Pseudomonas aeruginosa ATCC 27853*

Negative control- *Escherichia coli ATCC 25922*

Interpretation:

Strong positive – black colonies with dry crystalline consistency

Intermediate – black colonies with no crystalline consistency

Weak – Pink colonies with occasional darkening at centres.

Microtitre plate method[55]

Isolates from fresh agar plates were inoculated in TSB with 1% glucose and incubated for 24 hours at 37°C and then diluted (1 in 100) with fresh medium. 200µl of the diluted cultures was inoculated into individual wells of sterile polystyrene flat bottom tissue culture platesand controls were used(blank well, broth control, dye control and fixative control). The tissue culture plate was incubated for 48 hours at 37°C. after incubation , the contents of each well was gently removed by tapping the plates. The wells were washed with 200µl of PBS(pH- 7.2) to remove free floating planktonic bacteria. Biofilms formed by adherent bacteria were fixed with 2% sodium acetate and stained with 250µl of 1% crystal violet solution . The plates then incubated at room temperature for 20 minutes. Excess stain was rinsed off by thorough washing with 250µl of deionised water for 4 times. Adherent cells which usually formed biofilm on side walls was uniformly stained with crystal violet. Crystal violet stained biofilm was solubilised in 200µl of 95% ethanol

to extract the violet colour to quantify it. Optical densities of stained adherent bacteria was determined at the wavelength of 570nm using Spectrophotometer. These OD values were considered as the index of bacteria adhering to surface and forming biofilms.

Positive control- *Pseudomonas aeruginosa ATCC 27853*

Negative control- *Escherichia coli ATCC 25922*

Interpretation:

ODc(optical densitycut off value)= average OD of negative control + 3×SD(standard deviation) of negative control

Average OD value	**Biofilm production**
>4× ODc	Strong
<2× ODc - ≤4× ODc	Moderate
≤ 2× ODc	Weak / Negative

RESULTS

RESULTS

The study group included **105 patients** admitted in Medical, Surgical and Trauma Intensive Care Units with indwelling Central Venous Catheter. Among the study group,69(66%)were male patients and 36(34%)were female patients.

The patients comprising the study group were from 3 intensive care units in the distribution of

Medical Intensive Care Unit-34 patients (32%)

Surgical Intensive Care Unit -46 patients(44%)

Trauma Intensive Care Unit -25 patients(24%)

Among the 105 patients who were clinically suspected to have CRBSI, 16 patients had Laboratory Confirmed CRBSI . So the infection rate was 15.23%. The patients had CVC insertion either on an emergency indication (67 patients) or on an elective indication (38 patients). The commonest specific indication for catheterization was Lack of peripheral venous access among patients followed by Total parenteral nutrition along with blood transfusion, fluid replacement during surgery, to resuscitate the patient from shock, Central venous pressure monitoring and Dialysis.

The results obtained from each and every patient were consolidated and depicted in tables and charts as follows where

No. of patients (n) stand for the total number of patients with clinically suspected CRBSI [n=105] and **No. of CRBSI stands for the number of patients with laboratory confirmed CRBSI [n=16]**

Table 1 : Age and Gender wise distribution of study recruits with clinical suspicion of CRBSI (n=105)

Age group	Males(n=69)	Females(n=36)	Total(%)
18-29 years	9	9	18 (17)
30-39 years	15	9	24(23)
40-49 years	20	7	27(26)
50-59 years	12	5	17(16)
60-69 years	8	4	12(11)
70-80 years	5	2	7(7)
Total	69	36	105

26% of the study recruits belong to the age group of 40-49 years and most of them are male patients.

Chart1: Age and Gender wise distribution of study recruits with clinical suspicion of CRBSI(n=105)

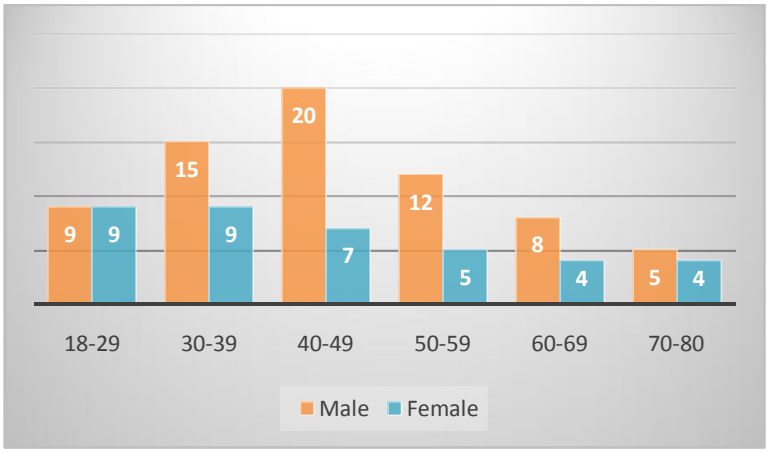


Table2 : Age wise distribution of study recruits(n=105)having Laboratory Confirmed CRBSI(N=16)

Age group	No. of patients(n=105)	No. of CRBSI(N=16) (%)
18-29 years	18	3(19)
30-39 years	24	2(12)
40-49 years	27	3(19)
50-59 years	17	5(32)
60-69 years	12	1(6)
70-80 years	7	2(12)
Total	105	16

32% of patients had laboratory confirmed CRBSI in the age group of 50-59 years when compared to other age groups.

Chart2 :Age wise distribution of study recruits(n=105)having Laboratory Confirmed CRBSI(N=16)

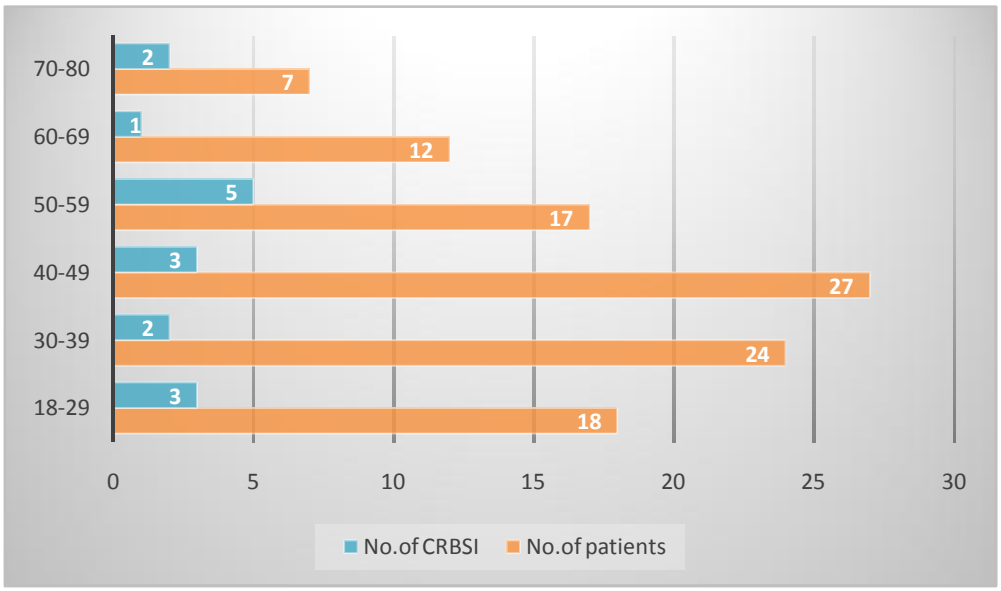


Table 3 : Distribution of patients having CRBSI in Intensive care units (n=105)

Intensive Care Unit	No. of patients(n=105)	No. of CRBSI (%)
Medical	34	6 (17.64)
Surgical	46	6 (13.04)
Trauma	25	4 (16)
Total	105	16 (15.23)

The overall incidence was found to be 15.23% .

Chart 3 :Distribution of patients having CRBSI in Intensive care units (n=105)

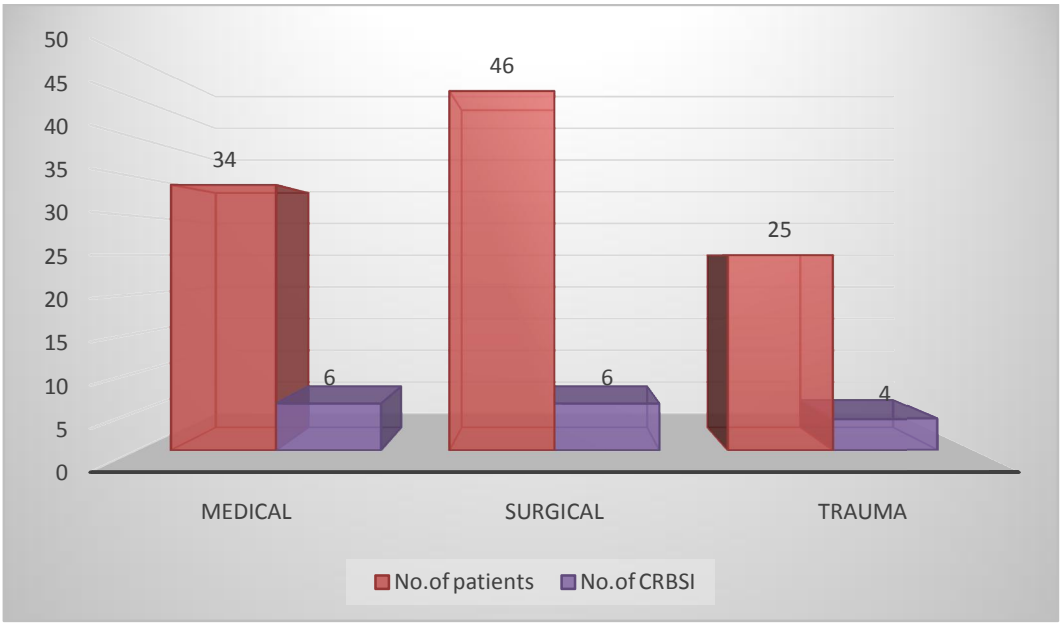


Table 4 : Correlation of the Emergency/Elective indication for CVC insertion with CRBSI(n=105)

Indication of CVC insertion	No.of patients	No. of CRBSI(%)
Emergency	67	13(81.25)
Elective	38	3(18.75)
Total	105	16

Emergency indication causes a higher rate of CRBSI (81.25%) and the difference is statistically significant (p = 0.006)

Chart 4 : Correlation of the Emergency/Elective indication for CVC insertion with CRBSI(n=105)

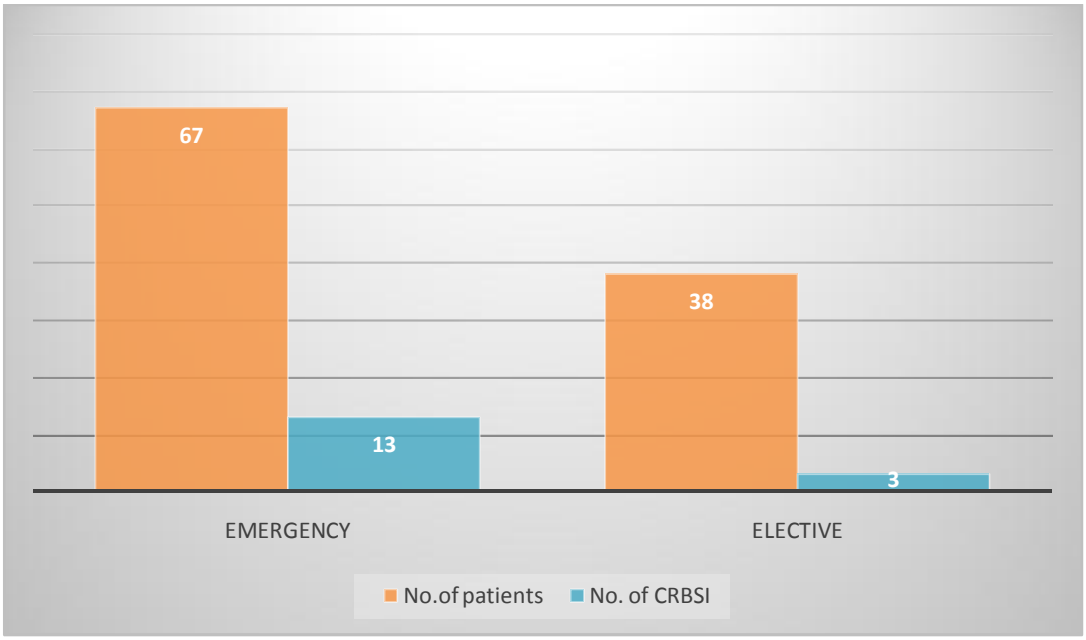


Table 5 :Distribution of the Specific Indications for CVC insertion with CRBSI(n=105)

Indication for CVC insertion	No.of patients (n=105)	No.ofCRBSI(%)
Fluid replacement during surgery	20	3(18.75)
To resuscitate the patient from shock	17	3(18.75)
Lack of peripheral venous access	27	4(25)
Blood transfusion and Total parenteral nutrition	23	4(25)
Central Venous Pressure monitoring	13	2(12.5)
Dialysis	5	- (0)
Total	105	16

Table 6 : Correlation of the site of CVC insertion with CRBSI(n=105)

Site of CVC insertion	No.of patients	No.ofCRBSI(%)
Subclavian site	52	7(13.46)
Jugular site	30	5(16.67)
Femoral site	23	4(17.39)
Total	105	16

The most common site for CVC insertion was Subclavian site whereas CRBSI rate was highest with femoral site of insertion(17.39%).

Chart 5: Correlation of the site of CVC insertion with CRBSI(n=105)

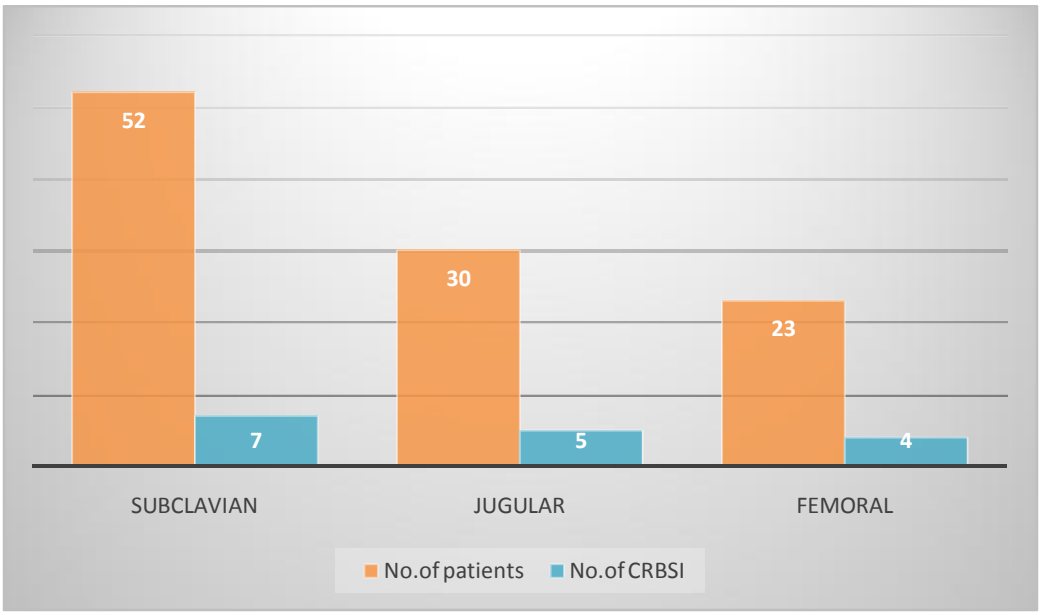


Table 7: Correlation of Duration of Catheterization with CRBSI

Intensive care unit	No.of days of catheterization	Total no.of patients	No.of patients (n=105)	No.of catheter days	No.of CRBSI (n=16)	Infection rate per 1000 device days
IMCU	≤ 7 days	23	14	79	1	12.65
	>7 days	29	20	319	5	15.67
ISCU	≤ 7 days	35	27	213	2	9.38
	>7 days	28	19	245	4	16.32
ITCU	≤ 7 days	16	10	68	1	14.70
	>7 days	26	15	202	3	14.48
TOTAL		157	105	1126	16	**14.20**

Incidence of CRBSI in patients with ≤ 7 days of catheterization in all three intensive care units was **7.84%**

Incidence of CRBSI in patients with >7 days of catheterization in all three intensive care units was **22.22%**

Infection rate per 1000 device days in patients with ≤ 7 days of catheterization in all three intensive care units was **11.11 per 1000 device days**

Infection rate per 1000 device days in patients with > 7 days of catheterization in all three intensive care units was **15.66 per 1000 device days**

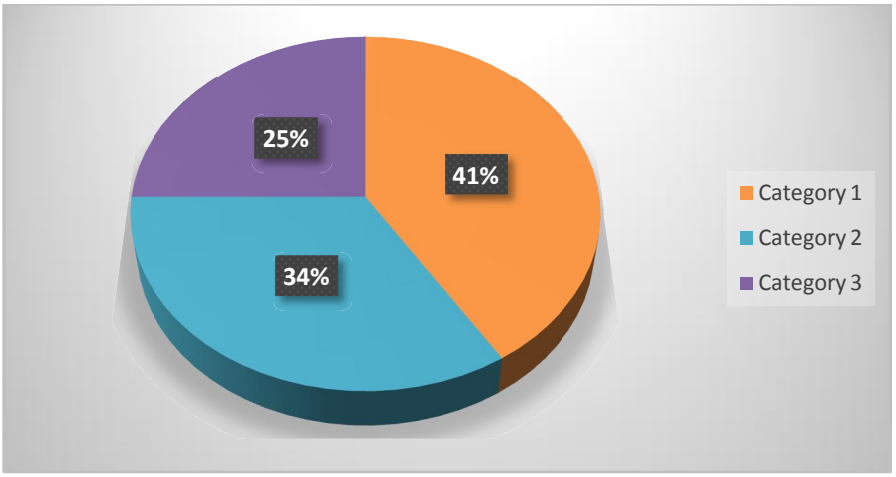
There was significant statistical difference between the incidence and infection rates in patients with ≤ 7 days of catheterization when compared with the incidence and infection rates in patients with > 7 days of catheterization **(p= 0.004)**

Table 8 : Distribution of the categories of sample collected (n=105)

Category of sample collected	No.of patients (%)	Samples collected		
		Catheter blood	Catheter tip	Peripheral blood
Category 1	43(41)	43	ND	43
Category 2	36(34)	ND	36	36
Category 3	26(25)	26	ND	ND
Total	105	181 samples collected		

ND – Not Done

Chart 6 :Distribution of categories of samples collected(n=105)



NOTE:

Category[1]-Patients with suspected CRBSI with maintenance Central venous catheterization

Category[2]-Patients with suspected CRBSI in whom maintenance catheterization is not indicated and central venous catheter can be removed

Table 9 : Culture positivity of Category 1 samples(n=43)

Culture positivity	Peripheral blood culture positive	Peripheral blood culture negative	Total
Catheter blood culture positive	7	15	22
Catheter blood culture negative	-	21	21
Total	7	36	43

Table 10 : Culture positivity of Category 2 samples(n=36)

Culture positivity	Peripheral blood culture positive	Peripheral blood culture negative	Total
Catheter tip culture positive	5	19	24
Catheter tip culture negative	-	12	12
Total	5	31	36

Table 11: Culture positivity of Category 3 samples(n=26)

Culture positive		Culture negative
≥100 cfu/ml	**<100 cfu/ml**	
4	7	15

Table 12 :Distribution of patients in IMCU(with CRBSI) with respect to their clinical diagnosis (n=34)

Clinical diagnosis	No.of patients(n=34)	No.ofCRBSI(%)
Acute CVA	9	2(33.33)
COPD with Type 2 Respiratory failure	5	1(16.67)
Post natal PPH with Acute renal failure	3	-(0)
DCLD with Hepatoencephalopathy	4	1(16.67)
Poisoning, Snake bite	6	1(16.67)
Autoimmune disorders	7	1(16.67)
TOTAL	34	6

Table 13 :Distribution of patients in ISCU(with CRBSI) with respect to their clinical diagnosis (n=46)

Clinical diagnosis	No.of patients (n=46)	Patients with CRBSI(%)
Intestinal obstruction	12	1(16.67)
Hollow viscus perforation	13	3(50)
Blunt injury abdomen	5	1(16.67)
Acute pancreatitis	6	-
Multinodular goitre thyroid	3	-
Splenectomy cases	3	-
Vascular injury cases	4	1(16.67)
TOTAL	46	6

Table 14 :Distribution of patients in ITCU(with CRBSI) with respect to their clinical diagnosis (n=25)

Clinical diagnosis	No.of patients	Patients with CRBSI(%)
RTA with Polytrauma	11	1(25)
RTA with head injury	5	1(25)
Polytrauma with vascular injury	3	1(25)
Hydrocephalus with VP shunt	4	
Self fall with Loss of consciousness	2	1(25)
TOTAL	25	4

Out of 105 patients with Central Venous Catheterization in the study group, bacteria were isolated from 15 patients (Laboratory Confirmed CRBSI) of which 11 were *gram negative* organisms(68.75%) and 4 were *gram positive* organisms(25%). *Candida albicans* was isolated from one patient(6.25%).

Table 15: Culture profile of microorganisms causing CRBSI(n=16)

Microorganisms	No.of isolates	Percentage
Pseudomonas aeruginosa	4	25
Klebsiellapneumoniae	3	18.75
Acinetobacterbaumanii	2	12.5
Klebsiellaoxytoca	1	6.25
Proteus vulgaris	1	6.25
Staphylococcus aureus	3	18.75
Staphylococcus epidermidis	1	6.25
Candida albicans	1	6.25
TOTAL	**16**	**100**

Chart 7: Culture profile of microorganisms causing CRBSI

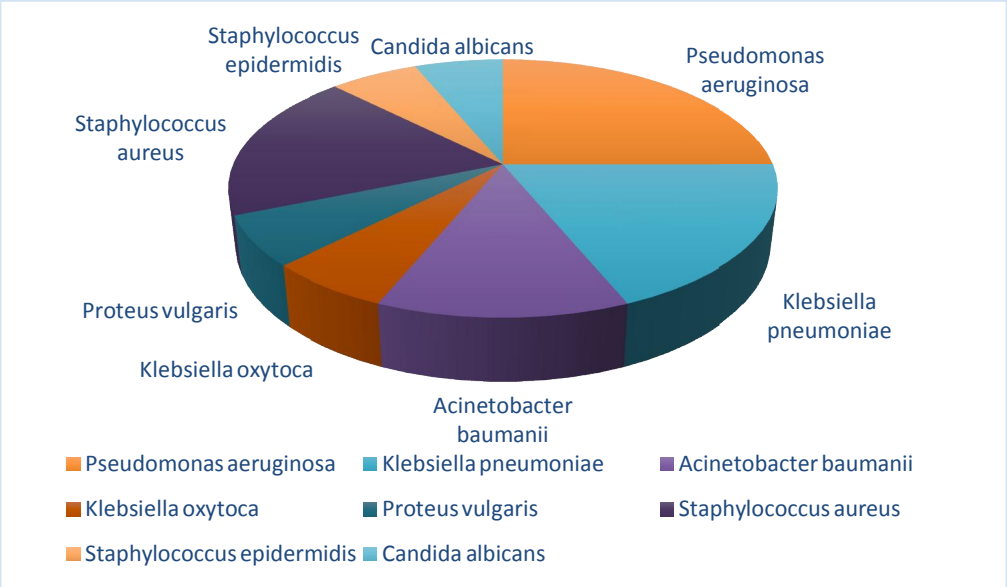


Table 16: Percentage of Antimicrobial Susceptibility Pattern in Gram negative organisms

Gram negative organism isolated (n=11)	AK %	GEN %	CIP %	COT %	TET %	IMI %	PT %	CZ %	CTX %
Pseudomonas aeruginosa (n=4)	75	25	25	NT	NT	100	100	25	NT
Klebsiellapneumoniae (n=3)	66	33	33	33	33	100	66	NT	0
Acinetobacterbaumanii (n=2)	50	0	0	100	50	100	100	0	NT
Klebsiellaoxytoca (n=1)	100	100	0	0	100	100	100	NT	0
Proteus vulgaris (n=1)	100	0	0	0	100	100	100	NT	0.

NT – Not Tested

AK - Amikacin

GEN - Gentamicin

CIP - Ciprofloxacin

COT - Cotrimozazole

TET - Tetracycline

IMI - Imipenem

PT - Piperacillin Tazobactum

CZ - Ceftazidime

CTX - Cefotaxime

Table 17: Antimicrobial susceptibility pattern of Gram positive organisms

Gram positive organism isolated	PEN	ERY	COT	TET	CIP	AK	GEN
Methicillin Resistant Staphylococcus aureus (n=2)	0	50	50	50	50	100	50
Methicillin sensitive Staphylococcus aureus (n=1)	0	0	100	100	100	100	100
Methicillin sensitive Staphylococcus epidermidis (n=1)	0	0	100	100	100	100	0

PEN - Penicilin

ERY - Erythromycin

AK - Amikacin

GEN - Gentamicin

CIP - Ciprofloxacin

COT - Cotrimozazole

TET - Tetracycline

Table 18: ESBL producers among *Enterobacteriaceae*(n=5)

Organism	No. of ESBL producers	Percentage
Klebsiellapneumoniae (n=3)	3	100%
Klebsiellaoxytoca (n=1)	1	100%
Proteus vulgaris (n=1)	1	100%

Table 19 : Sensitivity of *MRSA* isolates to Vancomycin by MIC

No.of*MRSA* isolates	Sensitive MIC < 2µg/ml	Resistant MIC> 2µg/ml
2	2	-

Table 20: Antifungal susceptibility pattern of *Candida albicans* by disc diffusion

Fungus isolated	Fluconazole	Voriconazole
Candida albicans (n=1)	100%	100%

Biofilm formation of the isolates were tested by three methods namely tube method, Microtitre plate method and Congo red agar method with the results depicted in tables and charts below

Table 21:Biofilm formation of different organisms by tube method

Organisms tested for biofilm	Strong biofilm formation(%)	Moderate biofilm formation(%)	Weak biofilm formation(%)
Pseudomonas aeruginosa (n=4)	25	-	75
Klebsiellapneumoniae (n=3)	33	33	34
Acinetobacterbaumanii (n=2)	-	100	-
Klebsiellaoxytoca (n=1)	-	100	-
Proteus vulgaris (n=1)	-	-	100
Methicillin Resistant Staphylococcus aureus (n=2)	50	50	-
Methicillin sensitive Staphylococcus aureus (n=1)	-	-	100
Methicillin sensitive Staphylococcus epidermidis (n=1)	-	-	100
Candida albicans (n=1)	-	-	100

Chart 8 : Biofilm formation by Tube method

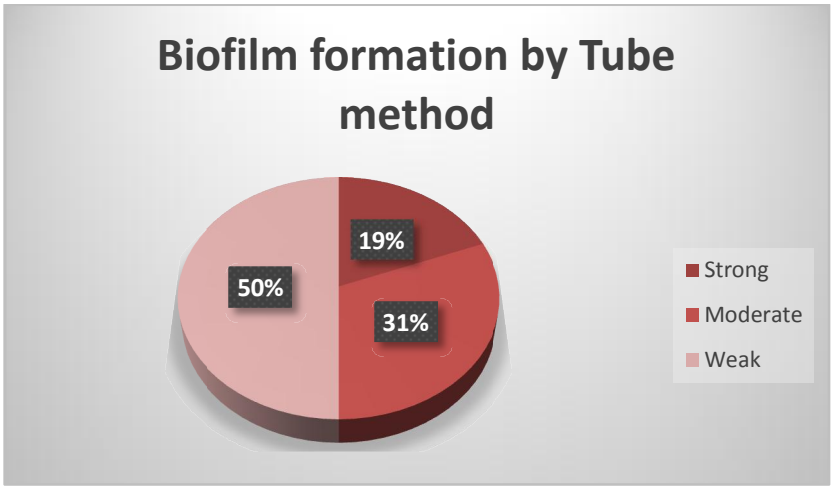


Table 22:Biofilm formation of different organisms by microtitre plate method

Organisms tested for biofilm	Strong biofilm formation(%)	Moderate biofilm formation(%)	Weak biofilm formation(%)
Pseudomonas aeruginosa (n=4)	25	25	50
Klebsiellapneumoniae (n=3)	67	33	-
Acinetobacterbaumanii (n=2)	100	-	-
Klebsiellaoxytoca (n=1)	100	-	-
Proteus vulgaris (n=1)	-	-	100
Methicillin Resistant Staphylococcus aureus (n=2)	100	-	-
Methicillin sensitive Staphylococcus aureus (n=1)	-	-	100
Methicillin sensitive Staphylococcus epidermidis (n=1)	-	-	100
Candida albicans(n=1)	-	-	100

Chart 9 : Biofilm formation by microtitre plate method

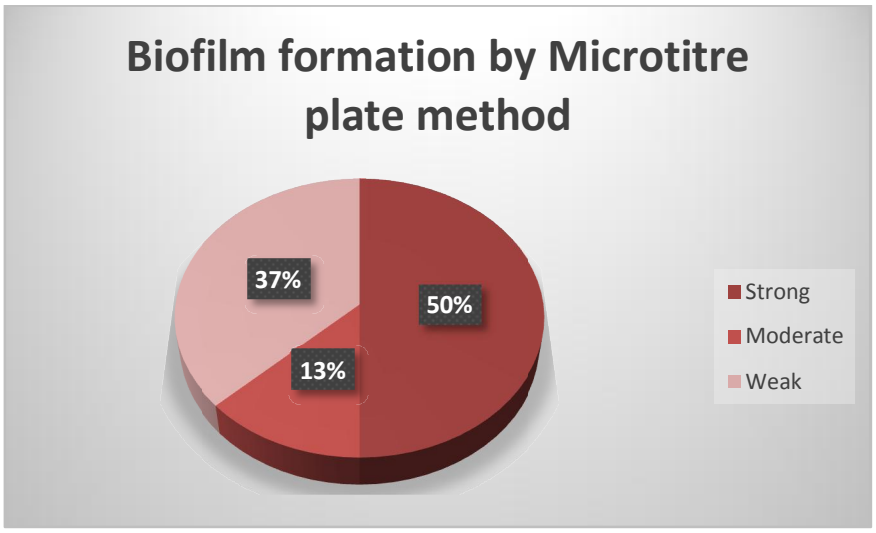


Table 23:Biofilm formation of different organisms by Congo red agar method

Organisms tested for biofilm	Strong biofilm formation(%)	Moderate biofilm formation(%)	Weak biofilm formation(%)
Pseudomonas aeruginosa (n=4)	25	50	25
Klebsiellapneumoniae (n=3)	67	33	-
Acinetobacterbaumanii (n=2)	100	-	-
Klebsiellaoxytoca (n=1)	100	-	-
Proteus vulgaris (n=1)	-	-	100
Methicillin Resistant Staphylococcus aureus (n=2)	100	-	-
Methicillin sensitive Staphylococcus aureus (n=1)	-	-	100
Methicillin sensitive Staphylococcus epidermidis (n=1)	-	-	100
Candida albicans(n=1)	-	-	100

Chart 10 : Biofilm formation by Congo red agar method

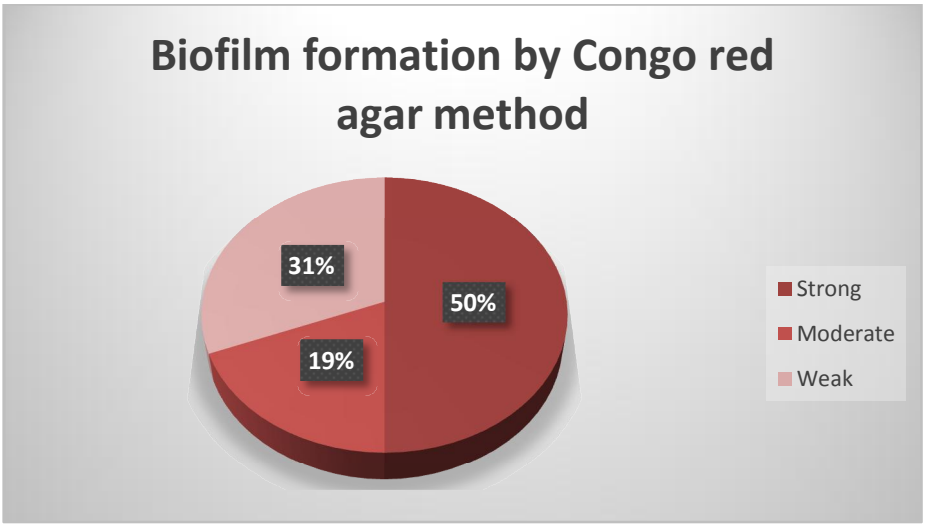


Table 24: Comparison of biofilm formation of different organisms by all three methods.

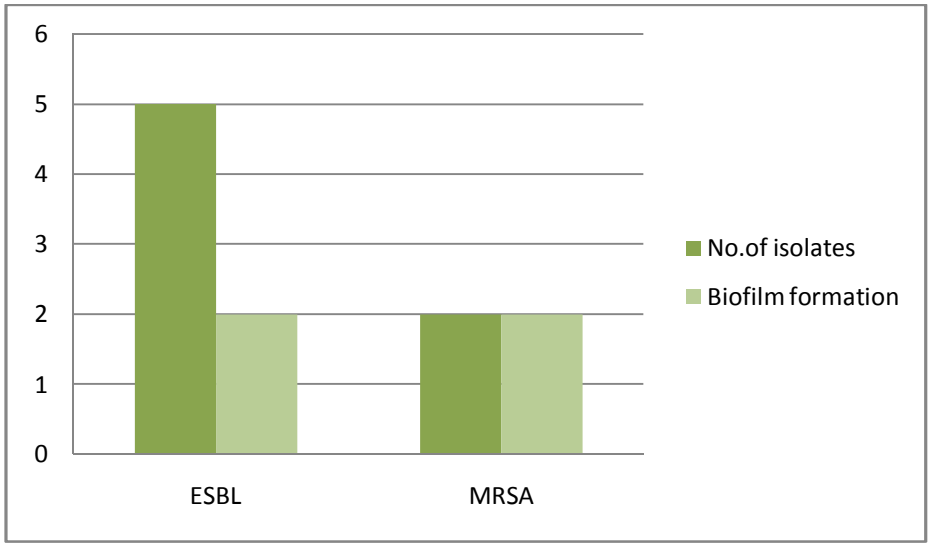
BIOFILM / METHOD	Strong biofilm formation	Moderate biofilm formation	Weak biofilm formation
Tube method	3	5	8
Microtitre plate method	8	2	6
Congo red agar method	8	3	5

Biofilm formation was considered to be similar between the microtitre plate method and congo red agar method while the tube method results vary with other two methods.This potential was correlated with the antimicrobial resistance of the organism.

Table 25: Correlation betweenantimicrobial resistance and biofilm formation

Antimicrobial resistance	No.of Biofilm producers (%)
Extended Spectrum beta lactamase production in Enterobacteriaceae (n=5)	3 (60)
Methicillin Resistance in Staphylococcus (n=2)	2 (100)

Chart 11: Correlation between antimicrobial resistance and biofilm formation



COLOUR PLATES

FIG : 1 – TRIPLE LUMEN CENTRAL VENOUS CATHETER

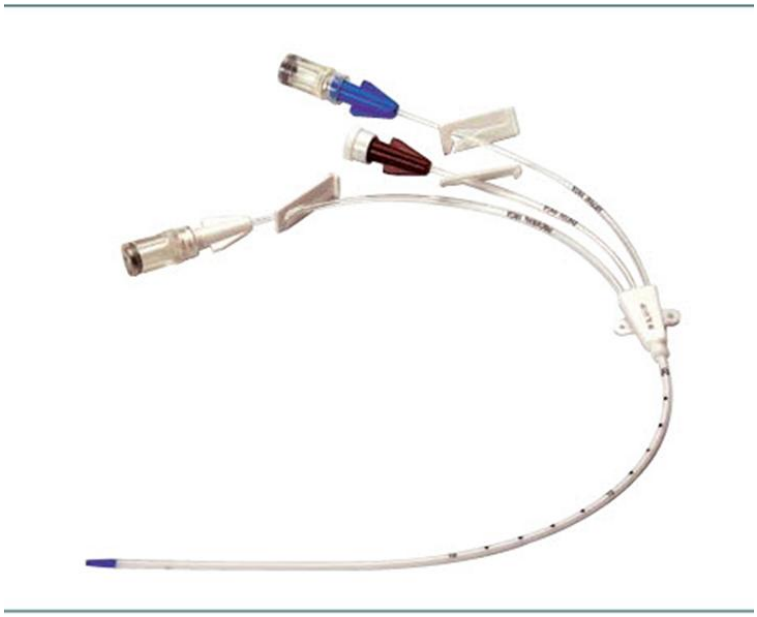


FIG : 2 – CATHETER TIP CULTURE –

ACINETOBACTER BAUMANII

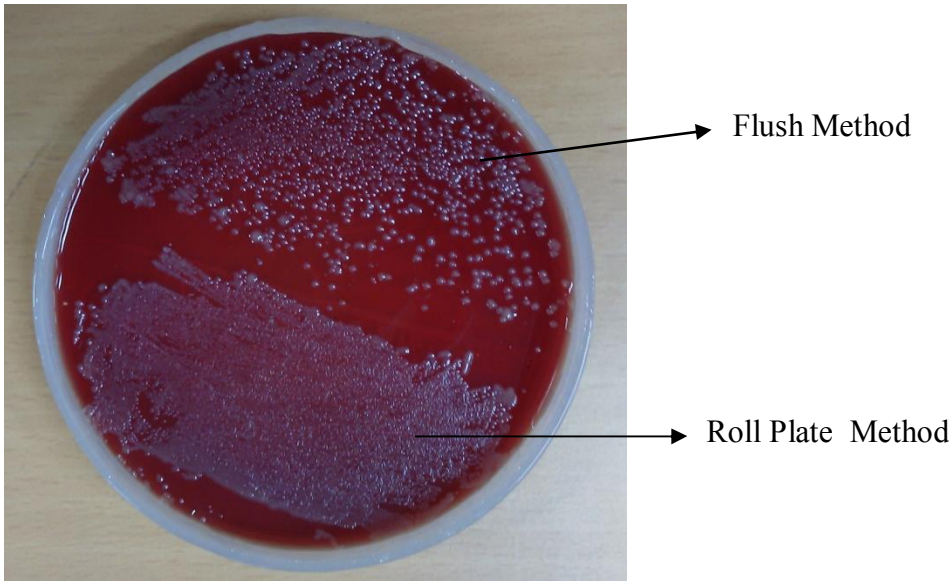


FIG : 3 – CATHETER TIP CULTURE –

PSEUDOMONAS AERUGINOSA

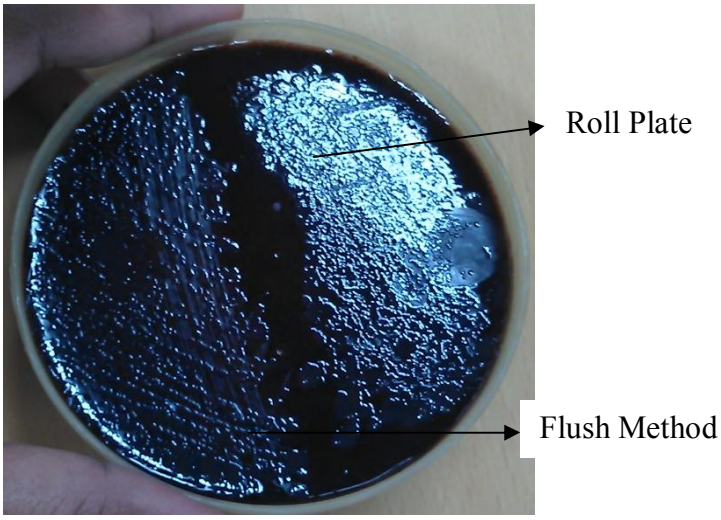


FIG : 4 – CATHETER BLOOD – POUR PLATE CULTURE

CANDIDA ALBICANS

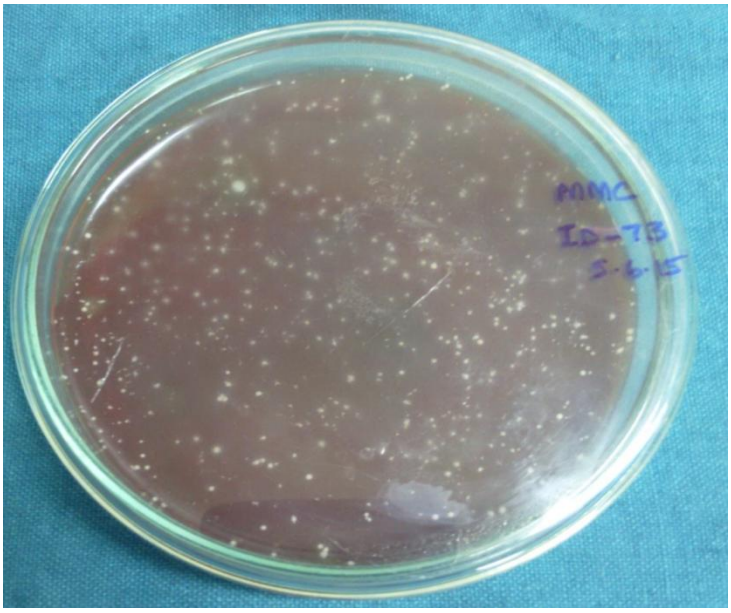


FIG : 5 – CANDIDA ALBICANS ON CHROME AGAR

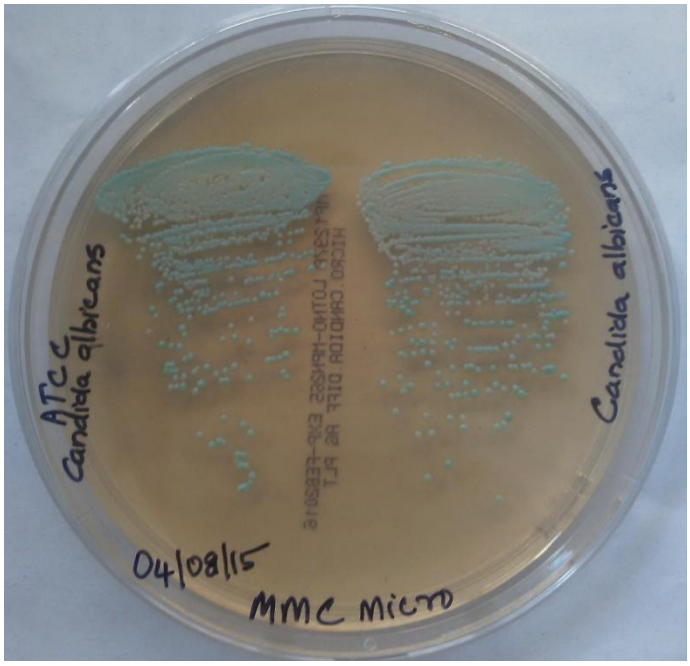


FIG : 6 – CANDIDA ALBICANS – GRAM'S STAIN

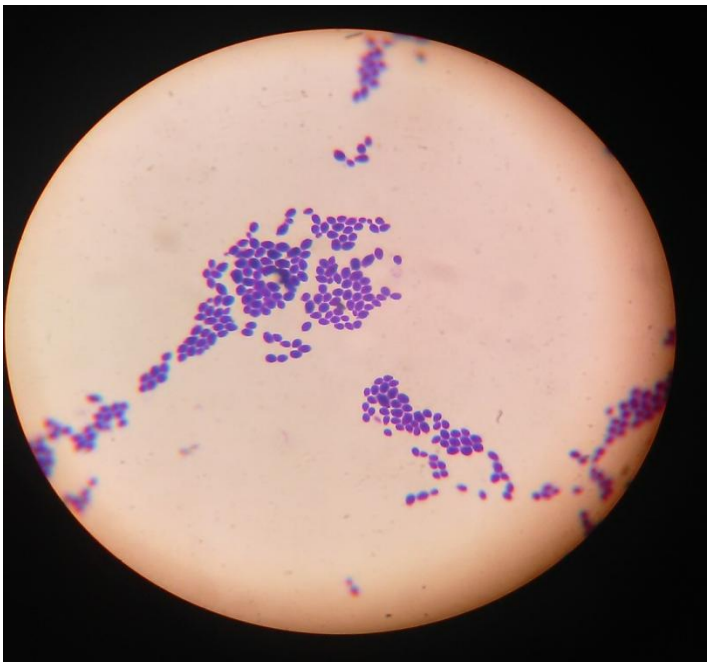


FIG : 7 – ANTIBIOGRAM OF MRSA SHOWING

CEFOXITIN RESISTANCE

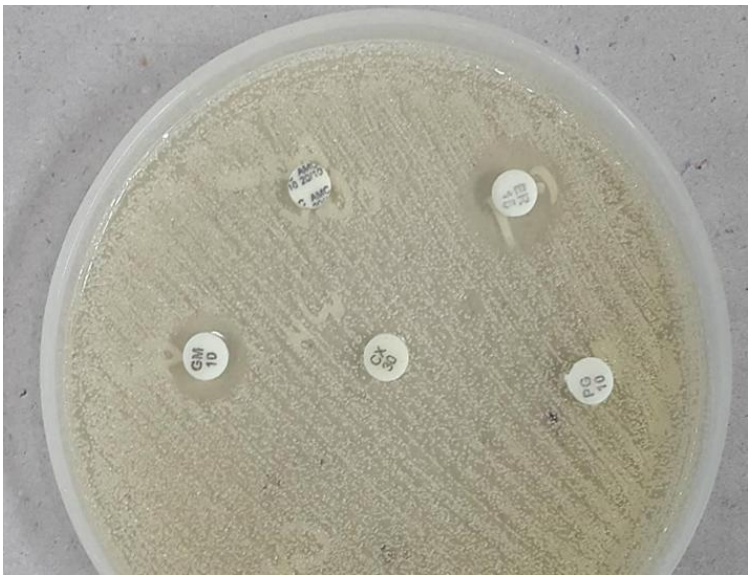


FIG : 8 – DETERMINATION OF VANCOMYCIN MIC BY E- STRIP

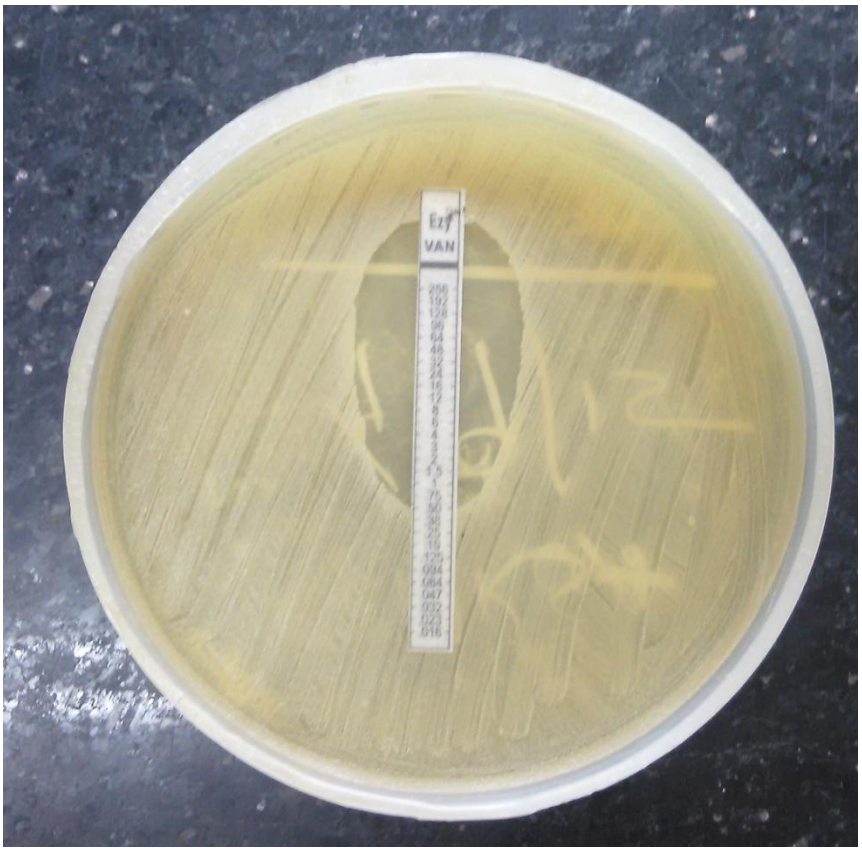


FIG : 9 – PHENOTYPIC CONFIRMATORY DISC DIFFUSION TEST FOR ESBL

FIG : 10 – PHENOTYPIC CONFIRMATORY DISC DIFFUSION TEST FOR ESBL

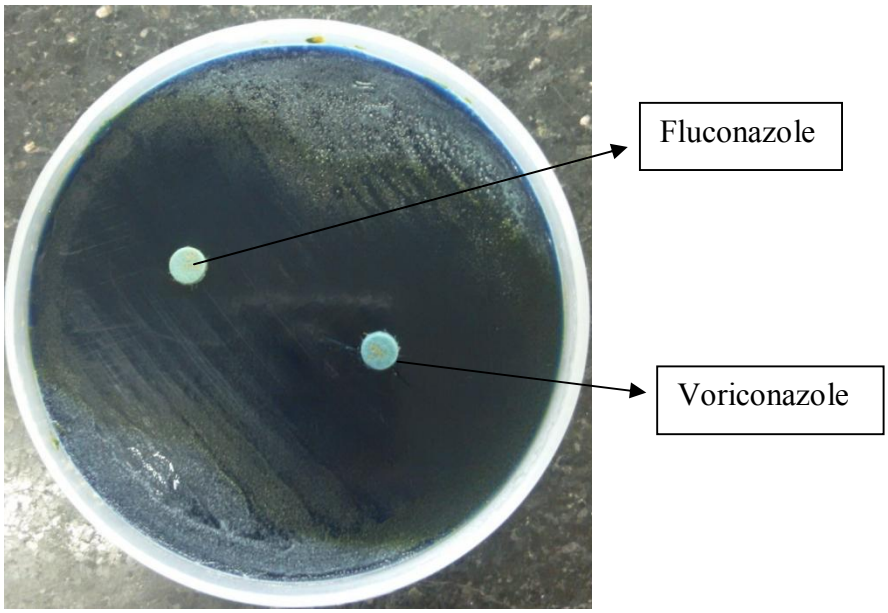


FIG : 11 – BIOFILM FORMATION BY TUBE METHOD

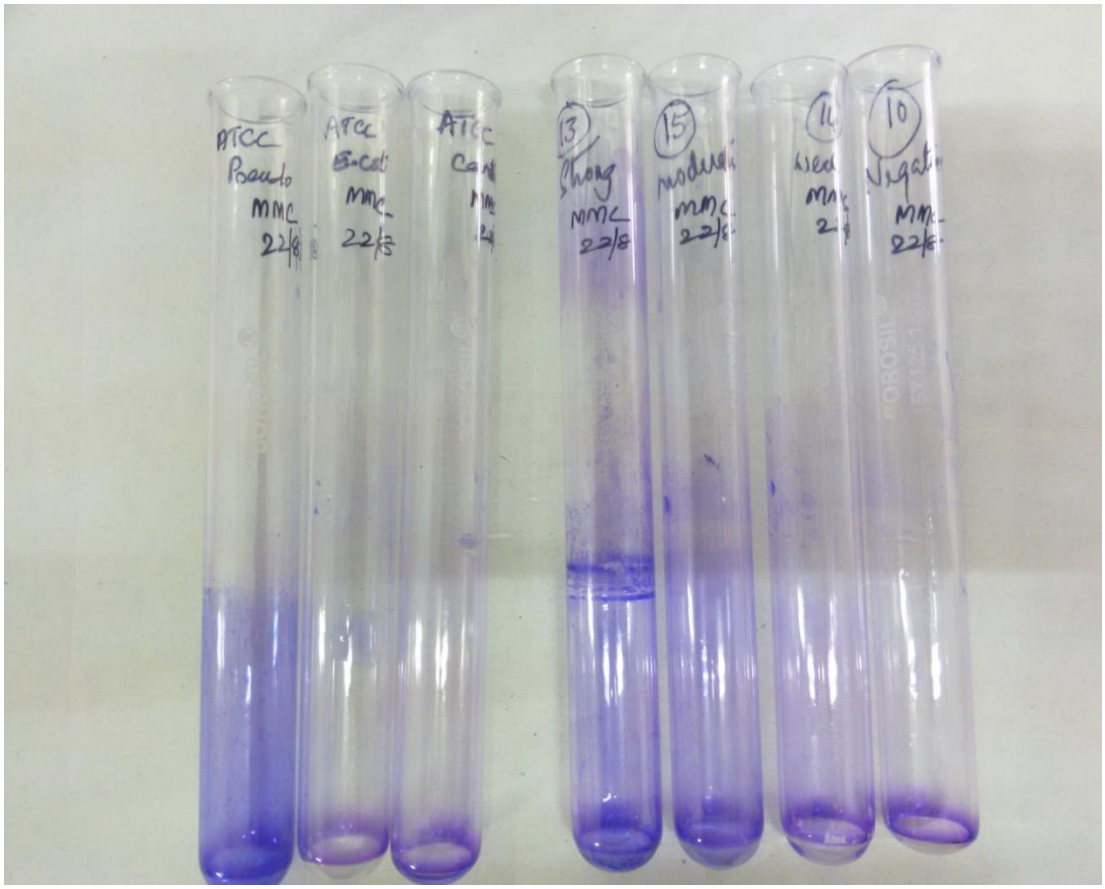


FIG : 12 – BIOFILM FORMATION BY MICROTITRE PLATE METHOD

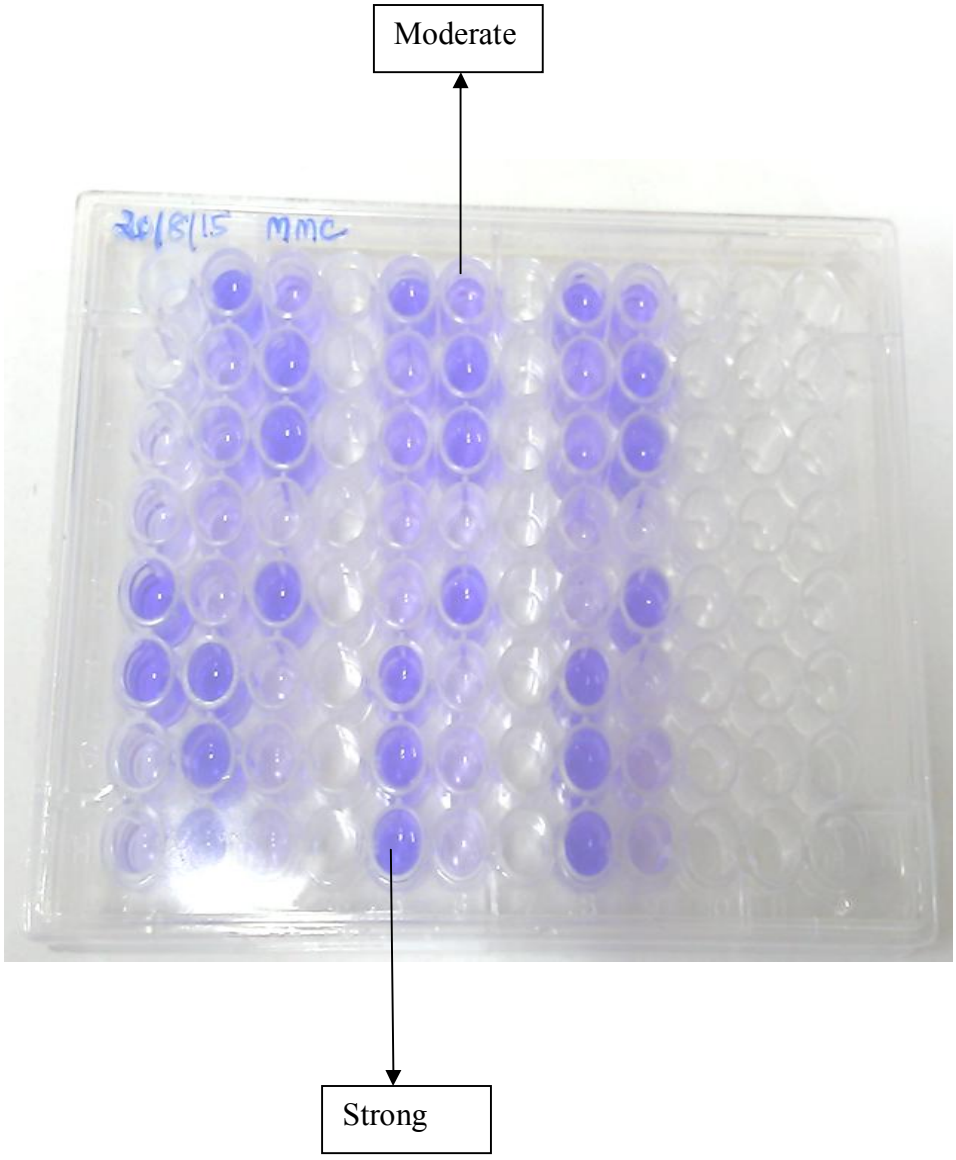
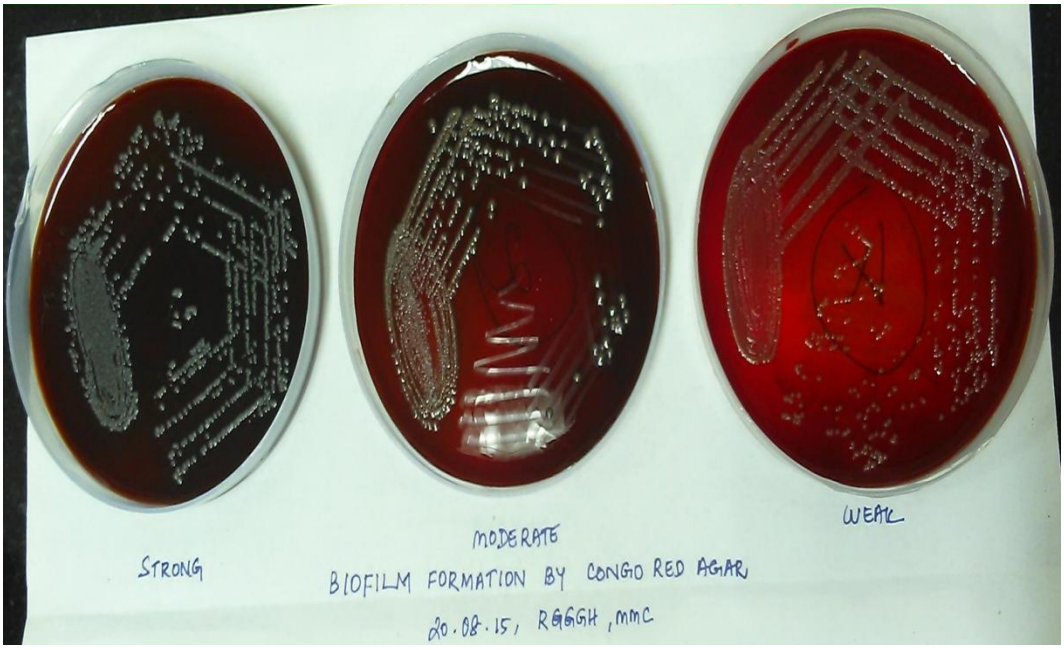


FIG : 13 – BIOFILM FORMATION BY CONGO RED METHOD



DISCUSSION

DISCUSSION

Intravenous catheter use poses an increased risk for blood stream infection of which Central venous catheter use stands out as the cause for the most dreadful hospital acquired infection in the intensive care units.The patients are at risk for acquiring the infection right from the admission of the patient in the ICU where the patients are exposed to the nosocomial pathogens and throughout the treatment process which is influenced by the hand hygiene practices of the hospital staff and the level of aseptic practices taken for CVC infusion. Hence my study was aimed to isolate and identify the microorganisms causing central venous catheter related blood stream infection along with their antimicrobial susceptibility pattern, to analyse the biofilm forming potential of the organisms isolated and to correlate the antimicrobial resistance with the biofilm formation. The data generated from the results of the study were analysed using SPSS version 21 software.

In this study including 105 patients from Intensive care units, 66% were males and 34% were females.The mean age in this study was 46.94.Similarily in a study conducted by Parameswaran et al in Karnataka, 66.3% males and 33.7% of females were included.[42] Similar results were also obtained from a study conducted by HarshaV.Patil et al in Maharastra where 59.25% were males and 40.74% were females.[56]

In my study,19% of the patients in the age group of 18-29 years, 12% of patients inthe age group of 30-39 years, 19% of patients inthe age group of

40-49 years, 32% of patients in the age group of 50-59 years, 6% of the patients in the age group of 60-69 years and 12% of the patients in the age group of 70-80 years developed CRBSI.

This study included only the Central Venous Catheters with three lumen. Maria Guembe et al at Spain performed a retrospective study in patients with microbiologically proven CRBSI in which all available catheter lumens were used to draw blood culture samples and studied 171 episodes of proven CRBSI in 154 patients.[57]

Out of 105 patients with clinical features of CRBSI in this study, 16 patients had laboratory confirmed CRBSI(15.23%) and the colonizer infection rate was 39.04%.

As with regards to the indication for CVC insertion in this study, the patients subjected to emergency mode of CVC insertion were 64% and those subjected to elective mode of CVC insertion were 36% which is statistically significant(p=0.006). The mode of insertion whether emergency or elective was decided by the clinical status of the patient at the time of admission. In this study, lack of peripheral venous access contributed to 26% of CVC insertion followed by Total Parenteral Nutrition and Blood transfusion 22%, Fluid replacement during surgery 19%, to resuscitate the patient from shock 16%, CVP monitoring 12% and dialysis 6%.

In this study, CRBSI rate was highest with femoral site insertion (17.39%),followed by jugular site insertion(16.67%) andsubclavian site insertion(13.46%).In a study conducted by Parameswaran et al in Karnataka, 33.3% of infections were caused by Femoral venous catheters, jugular venous catheters were responsible in 23% and subclavian catheters in 21.3%.[42]

There was significant statistical difference between the incidence and infection rates in patients with ≤ 7 days of catheterization when compared with the incidence and infection rates in patients with > 7 days of catheterization **(p= 0.004)**Incidence of CRBSI in patients with ≤ 7 days of catheterization in all three intensive care units was **7.84%**

Incidence of CRBSI in patients with >7 days of catheterization in all three intensive care units was **22.22%**

Infection rate per 1000 device days in patients with ≤ 7 days of catheterization in all three intensive care units was **11.11 per 1000 device days**

Infection rate per 1000 device days in patients with > 7 days of catheterization in all three intensive care units was **15.66 per 1000 device days**

In a study conducted by Harsha V.Patil et al in Maharastra, the infection rate of CRBSI was 47.31 per 1000 catheter days.[56]

Whereas the infection rate of CRBSI was very low 2.79 per 1000 catheter days in a study conducted by Randeep Kaur et al in Kerela.[59]In a study conducted by F.J.Mansur et al at Bangladesh, the infection rate of CRBSI was 16%.[61] In a study conducted by K.Chopdekar et al in Mumbai, average CRBSI rate was 9.26 per 1000 catheter days ranging from 8.64 per 1000 catheter days in PICU to maximum rate of 27.02 per 1000 catheter days in NICU.[58] In a study conducted by Min Chen et al at China, the catheter infection rate was 10.79% and CRBSI occurred 3.05 times per day per 1000 catheters.[62]

Among the isolates, Gram negative organisms accounted for 63%, Gram positive organisms for 31% and Candida albicans accounted for 6%.In a study conducted by Harsha V.Patil in Maharastra, Staphylococcus epidermidis was the commonest organism(50%). [56]

Of the Gram negative organisms, Pseudomonas aeruginosa (25%) was the commonest followed by Klebsiellapneumoniae (19%), Acinetobacterbaumanii (13%), Klebsiellaoxytoca(6%) and Proteus vulgaris (6%).

Of the Gram positive organisms, Staphylococcus aureus(19%) was the commonest followed by Staphylococcus epidermidis (6%) .

In a study conducted by Parameswaran et al in Karnataka, 64% of the CRBSI causing pathogens were Gram positive(commonly Staphylococcus

aureus) and 36% were Gram negative. Candida accounted for 10% of CRBSI.[42]A study conducted by Banda VenkataRamana et al in Andra Pradesh showed similar results to this study.The most common organism was Pseudomonas (30%), Klebsiellapneumoniae (18%), E.coli(16%), other Enterobacteriaceae(25%) and Non fermentative Gram Negative Bacilli(11%).[60]In a study conducted by F.J.Mansur et al at Bangladesh, most common pathogens were Pseudomonas sp (23.7%), Acinetobacter sp. (18.4%),Staphylococcus aureus(13.2%) and Enterobacteriaceae(10.5%). [61] In a study conducted by Stori et al in Brazil, 61.6% of infections were caused by gram positive organisms, 36.5% by gram negative organisms 9% by Candida species. [47]In a study conducted by Min Chen et al at China, 46.94% of gram negative bacilli and 40.14% of Gram positive cocci and 12.92% of Candida strains were isolated. [62]The varied bacterial profile and their incidence in CRBSI could be explained by the difference in the pattern of the nosocomial pathogens in each healthcare facility.

Analysis of Antimicrobial susceptibility showed that 75% of the Pseudomonas aeruginosaisolates were sensitive for Amikacin whereas only 25% of the isolates were sensitive each for Gentamicin, Ciprofloxacin and Ceftazidime. 100% of the isolates were sensitive for Imipenem and Piperacillin Tazobactum. This is similar to the study conducted by Banda Venkata Ramana et al in Andra Pradesh in which only 31% of isolates were sensitive to Ceftazidime.[60]

Among Klebsiellapneumoniae ,66% were sensitive to Amikacin and Piperacillintazobactum and all the isolates (100%) were sensitive to Imipenem. Only 33% of the isolates were sensitive each to Gentamicin, Ciprofloxacin, Cotrimoxazole and tetracyclines.

Among Klebsiella oxytoca,100% of the isolates were sensitive to Amikacin, Gentamicin, Tetracycline, Imipenem and Piperacillintazobactum whereas none of them were sensitive to ciprofloxacin and cotrimoxazole. All the Klebsiella (K.oxytoca and K.pneumoniae) isolates were resistant to Cefotaxime.

Among Acinetobacterbaumanii, 50%were sensitive to Amikacin and Tetracycline whereas 100% were sensitive to Cotrimoxazole, Imipenem and Piperacillintazobactum. All the isolates were resistant to Gentamicin , ciprofloxacin and ceftazidime.

Among Proteus vulgaris100% were sensitive for Amikacin, Tetracycline, Imipenem and Piperacillintazobactum whereas all of them were resistant to Gentamicin, Ciprofloxacin, Cotrimoxazole and Cefotaxime.

All the Enterobacteriaceaeisolates (100%) were ESBL producers. In a study conducted by Mansur et al at Bangladesh, rate of isolation of MRSA, Imipenem resistant Pseudomonas sp and ESBL producing Enterobacteriaceae were 60%, 44% and 100% respectively.[61] In a study conducted by

Parameswaran et al in Karnataka, 6.3% ESBL producers and 30.2% were Multidrug resistant organisms .[42]

Among the Staphylococcus aureus isolates,66% of the isolates were Methicillin resistant .In a study conducted by Parameswaran et al in Karnataka, 31% of MRSA were isolated.[42]A study conducted by Banda VenkataRamana et al in Andra Pradesh showed 65% of MRSA.[60]100% of the Methicillin sensitive Staphylococcus aureus isolates were sensitive to Amikacin, ciprofloxacin, tetracycline, cotrimoxazole and gentamicin whereas all were resistant to penicillin and erythromycin.100% of the Methicillin resistant Staphylococcus aureusisolates were sensitive to Amikacin whereas only 50% of the isolates were sensitive to erythromycin, cotrimoxazole, tetracycline, ciprofloxacin and gentamicin.100% of the Methicillin sensitive Staphylococcus epidermidisisolates were sensitive to Amikacin, Ciprofloxacin, cotrimoxazole and tetracycline whereas all the isolates were resistant to penicillin, erythromycin and gentamicin. 100% of Candida albicans were susceptible to fluconazole and voriconazole.

Regarding biofilm formation, the organisms tested for biofilm formation were Pseudomonas aeruginosa, Klebsiellapneumoniae, Klebsiellaoxytoca, Proteus vulgaris, Acinetobacterbaumanii, Staphylococcus aureus, Staphylococcus epidermidis and Candida albicans. In this study, among the gram negative organisms, 73% were biofilm producers and among

Gram positive organisms, 50% were biofilm producers. Biofilm production was demonstrated by tube method as Strong 18.75%, Moderate 25% and Weak 56.25% as compared with Microtitre plate method , in which 43.75%, 18.75% and 37.5% were Strong, Moderate and Weak respectively and also in Congo red agar method,50%, 12.5% and 37.5% were Strong, Moderate and Weak respectively.

Among the ESBL producers, 60% were biofilm producers and among the MRSA, 100% strong were biofilm producers suggesting a strong correlation (p=0.04) of biofilm production as a virulence factor in antimicrobial resistance.

Out of 16 patients with laboratory confirmed CRBSI, 14 patients recovered while 2 patients succumbed. The CRBSI mortality rate in my study is 12.5%, the infection rate of CRBSI is 14.2 per 1000 catheter days and CRBSI incidence is 15.23%.

SUMMARY

SUMMARY

The present study, ―A STUDY ON BIOFILM FORMATION IN ORGANISMS CAUSING CENTRAL VENOUS CATHETER ASSOCIATED BLOOD STREAM INFECTIONS IN INTENSIVE CARE UNIT PATIENTS IN A TERTIARY CARE HOSPITAL - was conducted at the Institute of Microbiology, Madras Medical College, RGGGH, Chennai-03.

It was a prospective study conducted for a period of one year from October 2014 to August 2015. The study included 105 patients from Intensive Medical, Surgical and Trauma care units with indwelling central venous catheter who developed symptoms of blood stream infections after 48hrs of catheterization.

Catheter segment (5cm tip) and peripheral and central venous blood samples were collected following aseptic precautions in clinically suspected patients with CRBSI and processed by standard microbiological methods forisolation, identification, biofilm formation and antimicrobial susceptibility testing of the isolates.

- Out of 105 clinically suspected patients, 16 patients had laboratory confirmed CRBSI(15.23%).
- Most common age group with CRBSI was between 50-59yrs

- Most common site of catheter insertion causing CRBSI was femoral site (17.39%)

- CRBSI was common with emergency mode of insertion (81.25%)

- Out of 16 patients with laboratory confirmed CRBSI, 14 patients recovered while 2 patients died. Thus the CRBSI mortality rate is 12.5% , the infection rate of CRBSI was 14.2 per 1000 catheter days and CRBSI incidence is 15.3%.

- In this study, 16 isolates were obtained for CRBSI in which Gram negative organisms accounted for 63% , Gram positive organisms accounted for 31% and Candida species accounted for 6%. Of the Gram negative organisms, Pseudomonas aeruginosa was the commonest 25% followed by Klebsiella pneumonia 19%, Acinetobacterbaumanii 13%, Klebsiellaoxytoca 6% and Proteus vulgaris 6%. Of the Gram positive organisms, Staphylococcus aureus was the commonest 19% followed by Staphylococcus epidermidis 6% . Candida albicans contributed to 6% of CRBSI.

- All the gram negative isolates were sensitive to Imipenem and all the Gram positive isolates obtained were sensitive to Vancomycin.

- Biofilm production was demonstrated by tube method as Strong 18.75%, Moderate 25% and Weak 56.25% as compared with Microtitre plate method , in which 43.75%, 18.75% and 37.5% were

Strong, Moderate and Weak respectively and also in Congo red agar method,50%, 12.5% and 37.5% were Strong, Moderate and Weak respectively.

- In this study, among the gram negative organisms, 73% were biofilm producers and among Gram positive organisms, 50% were biofilm producers.

- 100% of the Enterobacteraceae isolates were ESBL producers.66% of the isolates were Methicillin resistant Staphylococcus aureus.

- Among the ESBL producers,60% were biofilm producers. Among the MRSA, 100% were biofilm producers.

CONCLUSION

CONCLUSION

Central venous catheters have unquestionable benefits in current medical practice, but their potential complications are also well known. One of the main complications is catheterrelated bloodstream infection (CRBSI). Occurrence of these infections in a hospital set up leads to decrease in the quality of health care provided to the patients. These infections often result in prolonged hospital stay, thereby exposing the patients to the risks of acquiring multiple infections which in turn leads to increase in morbidity as well as mortality.

The present study aimed at diagnosing such infections and identifying the potential pathogens implicated, in order to provide better patient care and to prevent such infections from occurring in the future.

The CRBSI rates in this study are slightly on the higher side when compared to other studies from India and however the incidence density is significantly on the higher side when compared with foreign studies, indicating the need for more stringent catheter care protocol.

In this study, gram negative organisms are implicated more in the causation of CRBSI and are also shown to produce strong biofilms than gram positive organisms, suggesting that biofilm formation is crucial in the pathogenesis of such infections.

The study showed the predominance of common nosocomial pathogens, MRSA and P.aeruginosa in the causation of these infections, thereby indicating that occurrence of such infections could be controlled by active surveillance strategies. The antibiogram of isolates causing CRBSI and those forming biofilm in this study clearly

indicated the increased resistance pattern to routinely used antibiotics among these isolates and that irrational usage of antibiotics have paved way for such rising resistance trends with biofilms accentuating the transfer of resistance genes from one organism to another. The antibiotic resistance pattern of the isolates in this study also suggested that Imipenem and Vancomycin are excellent drugs to combat infections and are still efficient in treating infections, even with those capable of producing biofilm and causing bloodstream infections in critical care setting.

The study concluded that CRBSI are on the rise due to increased interventions, and that they are caused by common nosocomial pathogens which are still sensitive to reserve drugs and to very few routine drugs, and that such infections can be controlled and prevented if strict catheter care techniques, rational use of available antibiotics and robust surveillance measures are followed.

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APPENDIX

APPENDIX I

LIST OF ABBREVIATIONS

ATCC – American Type Culture Collection

ALT – Antibiotic Lock Therapy

BHI – Brain-Heart Infusion

BSI – Blood Stream Infection

CRBSI – Catheter Related Blood Stream Infection

CDC – Center for Disease Control and Prevention

CLABSI – Central Line Associated Blood Stream Infection

CVC – Central Venous Catheter

CLSI – Clinical Laboratory Standards Institute

CONS – Coagulase Negative Staphylococci

CFU – Colony Forming Units

ELT – Ethanol Lock Therapy

EPS – Extracellular Polymeric Substance

HICPAC – Healthcare Infection Control Practices Advisory Committee

HAI – Hospital Acquired Infection

IDSA – Infectious Diseases Society of America

ICU – Intensive Care Unit

IVC – Intravenous Catheter

LCBI – Laboratory Confirmed Bloodstream Infection

MRSA – *Methicillin ResistantStaphylococcus aureus*

MSSA – *Methicillin SensitiveStaphylococcus aureus*

MR- Methyl Red test

ml – Millilitre

µg – Microgram

MHA -Mueller Hinton Agar

NHSN – National Health Safety Network

NICU – Neonatal Intensive Care Unit

NLF – Non lactose Fermenting

OF –Oxidation and Fermentation

PICC – Peripherally Inserted Central Venous Catheter

Spp –Species

TPN – Total Parenteral Nutrition

TSI –Triple Sugar Iron

VP – VogesProskauer test

APPENDIX II

A.STAINS AND REAGENTS

I. Gram staining

Methyl violet (2%)	l0g Methyl violet in l00ml absolute alcohol in 1litre of distilled water (primary stain)
Grams Iodine	l0g Iodine in 20g KI (fixative)
Acetone	Decolourising agent
Carbolfuchsin 1%	Secondary stain

B.MEDIA USED:

1.MacConkey agar

Peptone	20g
Sodium taurocholate	5g
Distilled Water	1 ltr
Agar	20g
2% neutral red in 50% ethanol	3.5ml
10% lactose solution	l00mI

Dissolve peptone and taurocholate in water by heating. Add agar and dissolve it in steamer. Adjust pH to 7.5. Add lactose and neutral red shake well and mix. Heat in free steam (100°C) for 1 hour, then autoclave at 115°C for 15minutes.

2. Blood agar (5% sheep blood agar)

Peptone	l0g
NaCl	5g
Distilled water	1 Ltr
Agar	10g

Dissolve ingredients in distilled water by boiling, and add 5% sheep blood(sterile) at 55°C adjust pH to 7.4.

3. Chocolate agar

Sterile defibrinated blood	10 ml
Nutrient Agar (melted)	100 ml

When the temperature was about 75°C, sterile blood was added with constant agitation. After addition of blood, kept in water bath and heating was continued till the blood changed to chocolate colour. Cooled to about 50° C and poured about 15ml into petri dishes with sterile precaution**.**

4. Mueller Hinton Agar:

Beef, infusion	300ml
Casein acid hydrolysate	17.5g
Starch	1.5g
Agar	10g
Distilled water	1 litre

Final pH (at 25°C) 7.3±0.1

Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

5.TRYPTIC SOY BROTH

This is reconstituted from the dehydrated form according to the manufacturer's instructions. For adult clinical practice, the reconstituted broth is distributed in 20 ml amounts in 30 ml medical flat bottles with perforated screw-caps and rubber liners. Autoclave with caps tight at 121^0C for 20 minutes. The exposed area of the liner in the perforation should be covered with a foil cap added before autoclaving.

C.MEDIA AND REAGENTS REQUIRED FOR BIOCHEMICAL IDENTIFICATION:

1.Oxidase Reagent

Tetra methyl p-phenylenediaminedihyrochloride- 1% aqueous solution.

2.Catalase test

3% hydrogen peroxide

3.Indole test

Kovac's reagent

Amyl or isoamyl alcohol	150ml
Para dimethyl amino benzaldehyde	10g
Concentrated hydrochloric acid	50ml

Dissolve the aldehyde in the alcohol and slowly add the acid. Prepare in small quantities and store in the refrigerator. Shake gently before use.

4. Christensen's Urease test medium

Peptone	1g
Sodium chloride	5g

Dipotassium hydrogen phosphate	2g
Phenol red	6ml
Agar	20g
Distilled water	1 ltr
10% sterile solution of glucose	l0ml
Sterile 20% urea solution	l00ml

Sterilize the glucose and urea solutions by filtration. Prepare the basal medium without glucose and urea, adjust to pH 6.8-6.9 and sterilize by autoclaving in a flask at 121°C for 30min. Cool to about 50°C, add the glucose & urea, and tube the medium as slopes.

5. Simmon's Citrate Medium

Koser's medium	1 ltr
Agar	20g
Bromothymol blue	0.2% 40ml

Dispense, autoclave at 121°C for 15 min and allow to set as slopes

6.Triple Sugar Iron medium

Beef extract	3g
Yeast extract	3g
Peptone	20g
Glucose	1g
Lactose	l0g
Sucrose	l0g
Ferric citrate	0.3g

Sodium chloride	5g
Sodiumthiosulphate	0.3g
Agar	12g
Phenol red 0.2% solution	l2ml
Distilled water	1 ltr

Heat to dissolve the solids, add the indicator solution, mix and tube.Sterilize at 121°C for 15 min and cool to form slopes with deep butts.

7.Glucose phosphate broth

Peptone	5g
Dipotassium hydrogen phosphate	5g
Water	1 ltr
Glucose 10% solution	50ml

Dissolve the peptone and phosphate and adjust the pH to 7.6. Filter dispense in 5ml amounts and sterilize at 121°C for 15min. Sterilize the glucose solution by filtration and add 0.25ml to each tube

Methyl Red Reagent

Methyl Red	10mg
Ethyl alcohol	30ml
Distilled water	20ml

VogesProskauer Reagent

Reagent A:

Alpha naphthol	5g
Ethyl alcohol	100ml

Reagent B:

Potassium hydroxide	40g
Distilled water	100ml

8. Peptone water sugars fermentation test medium.

To the basal medium of peptone water, add sterilised sugars, 1%indicator bromothymol blue with Durham's tube.

Basal medium peptone water

Sugar solutions:

Sugar	1ml
Dislilled water	l00ml

pH = 7.6.

9. Potassium nitrate broth

Potassium nitrate (KN03)	0.2gm
Peptone	5.0gm
Distilled water	100ml

The above ingredients were mixed and transferred into tubes in 5 ml amount and autoclave.

10.Decarboxylase media:

10a.Moeller decarboxylase broth base:

Ingredients	**gms/L**
Peptone	5
Beef extract	5

Bromocresol purple	0.01
Cresol red	0.005
Glucose	0.5
Pyridoxal	0.005

Final pH 6

10b. Aminoacid:

Add 10 g of the levo form of the amino acid for 1000ml.mix and dispense in sterile tubes.

11. Hugh&Leifson's Oxidation –Fermentation test:

Peptone	2g
Sodium chloride	5g
D-glucose	10g
Bromothymol blue	0.03g
Agar	3.0g
Dipotassium phosphate	0.30g
Distilled water	1L

pH =7.1

Basal medium is autoclaved.1% of sterile sugar solutions is added to the basal medium. Dispense into sterile test tubes without slant.

12. Phenolphthalein diphosphate agar

Sterilize 1% aqueous solution of sodium phenolphthalein diphosphate by filtration and store at 4ºC.Add 10ml of this solution to 1000ml melted nutrient agar cooled to 50ºCand pour plates. Grow the staphylococcus

overnight at 37ºC on the medium. Invert the plate and pour a few drops of ammonia solution SG 0.88 in to the lid. Read as positive a culture whose colonies turn bright pink within a few minutes. The colour soon fades.

13.Mannitol motility medium:

Peptone 20 g

Mannitol 2 g

Potassium nitrate 1 g

Agar 3 g

Phenol red 0.04 g

Suspend 26 g of the medium in 1000 ml of distilled water. Heat to boiling to dissolve completely. Dispense in tubes and sterilize by autoclaving.

APPENDIX III

Identification Tests used for the Isolates

GRAM STAINING

Procedure:

1. Smear is prepared and heat fixed.
2. Cover the smear with Gentian violet for 1 minute.
3. Wash and cover with Gram's iodine for 1 minute.
4. Wash and decolorize with acetone for 2 seconds.
5. Wash and counterstain with dilute carbolfuchsin for 30 seconds.
6. Wash with water, dry and observe under oil immersion.

MOTILITY BY HANGING DROP PREPARATION

Procedure:

1. A hollow ground glass slide with a shallow circular concavity is taken.
2. The concavity is encircled with soft petroleum jelly with the help of a stick.
3. A drop of the suspension of the culture is placed on the coverslip.
4. The slide is then inverted over the coverslip so that the drop hangs into the centre of concavity of the slide.
5. Examine the drop first with low power objective to view the edge of the drop and then with the high power to look for motile bacteria.

CATALASE TEST

Principle: This test demonstrates the presence of catalase, the enzyme that catalyzes the release of oxygen from hydrogen peroxide.

Procedure: A small amount of the colony to be tested is picked with a sterile thin glass rod and it is inserted into 3% hydrogen peroxide solution held in a small, clean tube.

Interpretation: Productionof gas bubbles was interpreted as positive catalase test and no bubble formation was interpreted as negative.

COAGULASE TEST

Principle: This test demonstrates the presence of enzyme coagulase produced by the organism.

SLIDE COAGULASE TEST

Procedure: A clean slide was taken and two portions made on it with a glass marking pencil. A drop of normal saline was placed on each portion. A portion of the colony was taken with the help of a loop and was emulsified in each of the two drops. A loopful of undiluted plasma was added to one of the suspensions. Clumping was seen if the strain was coagulase positive.

The control slide should not show any clumping.

TUBE COAGULASE TEST

To 1.0 ml of 1 in 6 plasma diluted in saline (0.85%), 0.1 ml of broth culture of the *Staphylococci* was added and incubated at 37 °C for 2 4 hours.

If the test is positive, due to the formation of the coagulum, the fluid does not flow when the tube is tilted. If it was negative at the end of 4 hours, it was left overnight at room temperature and the reading taken next morning.

CONTROL: Coagulase positive: *Staphylococcus aureus*

Coagulase negative*: Staphylococcus epidermidis*

OXIDASE TEST

Principle: This test demonstrates the presence of cytochrome oxidase enzyme which catalyses oxidation of cytochrome by oxygen.

Reagent: 1% Tetramethyl-*p*-phenylene-diaminedihydrochloride.

Procedure: Few drops of the freshly prepared 1% reagent were added to a strip of filter paper. A speck of culture was rubbed on it using a sterile glass rod.

Interpretation: A positive reaction was indicated by a dark purple colour appearing within

10 seconds.

Positive control: *Pseudomonas aeruginosa*

Negative control: *Escherchia coli*

Gram negative bacilli, both lactose fermenters and non-lactose fermenters, were subjected to the following tests.

A single colony of the organism was inoculated into peptone water and incubated for about 2 – 4 hours at 37 °C. Material from this was used for the biochemical tests.

INDOLE PRODUCTION TEST

Principle: This test demonstrates the presence of enzyme tryptophanase that degrades tryptophan to indole.

Procedure:

The test organism is inoculated into peptone water and incubated for 24 hours. 0.5 ml of Kovac's reagent is added to overnight broth.

Interpretation: Formation of a pink colour ring indicates a positive test.

METHYL RED TEST

Principle: This test detects the production of acid during the fermentation of glucose and maintenance of pH below 4.5.

Procedure: The test organism is inoculated into glucose phosphate medium and incubated at

37 ^{0}C for 48 to 72 hours. Few drops of 0.04% solution of methyl red is added to the culture.

Interpretation: Development of red colour is interpreted as a positive test.

VOGES-PROSKAUER TEST

Principle: This test detects the production of acetyl methyl carbinol from pyruvic acid as an intermediate stage in its conversion to 2,3 butylene glycol. In presence of alkali and atmospheric oxygen, small amount of acetyl methyl carbinol is oxidized to diacetyl which reacts with peptone in the broth.

Procedure: The test organism is inoculated into glucose phosphate medium and incubated at

37 °C for 48 to 72 hours. 0.6 ml of 5% solution of alpha-naphthol in ethanol and 0.2 ml of 40% KOH is added to 1 ml of culture.

Interpretation: Development of pink colour within 30 mins is interpreted as a positive test.

CITRATE UTILISATION TEST:

Principle: Some bacteria can obtain energy by utilising citrate as the sole source of carbon.

The utilisation of citrate was detected in Simmon's citrate medium by the production of alkaline by products.

Procedure: The entire surface of the slant was inoculated lightly from a young culture and incubated at 37 °C for 24-48 hours.

Interpretation: The test was considered positive when the medium turned deep blue in colour along with growth on the surface.

Positive Control: *Klebsiellapneumoniae*

Negative Control: *Escherchia coli*

UREASE TEST:

Principle: Bacteria possessing urease enzyme hydrolyze urea, producing ammonium bicarbonate and CO2 resulting in alkalinisation and increase in pH of the medium.

Sterilize the glucose and urea solutions by filtration. Prepare the basal medium without glucose or urea. Adjust pH to 6.8 – 6.9 and sterilize by autoclaving in a flask at 121 °C for 15 minutes. Cool to about 50 °C. Add the glucose and the urea solutions and tube the mediumas deep slopes.

Procedure: The medium was inoculated with the test organisms and incubated at 37 °C for 24 – 48 hours.

Interpretation: Development of pink colour throughout the medium was a positive test; yellow colour was a negative test.

Positive control: *Proteus vulgaris*

Negative control: *Escherchia coli*

MANNITOL MOTILITY TEST

In semi-solid agar medium, motile bacteria ―swarm‖ and give a diffuse spreading growth that is easily recognized by the naked eye. Motility may thus be detected more easily than by microscopical ―hanging drop‖ method

Procedure: The centre of butt of the MMM was stabbed with a straight wire charged with a young culture incubated at 37 °C overnight.

TRIPLE SUGAR IRON AGAR (TSI)

Principle: This detects the fermentation of the three sugars (namely glucose, lactose and sucrose), the production of gas and hydrogen sulphide. Lactose and sucrose are present in a concentration ten times that of glucose. Phenol red and ferrous sulphate serve as indicators of acidification and H2 S formation, respectively.

Adjust the pH to 7.6. Then add phenol red 0.24 g. Distribute into small test tubes in 4ml quantities and autoclave at 121 °C for 15 minutes. Keep the tubes in a slanting position immediately after that, so as to get a butt &slan of equal proportions.

Procedure: The centre of butt of the TSI was stabbed with a straight wire charged with a young culture and the slant was streaked and incubated at 37 °C overnight for 18 to 24 hours.

Interpretation:

Reaction	Explanation
Acid butt (yellow) Alkaline slant (red)	Glucose fermented
Acid butt (yellow) Acid slant (yellow)	Glucose and lactose/or sucrose fermented

Gas bubbles in butt	Aerogenic cultures indicate production of CO2 or H2
Medium sometimes split, blackening in butt	Hydrogen sulphide produced
Alkaline slant and butt (entire medium red)	Glucose, lactose and sucrose nonutilizers

SUGAR FERMENTATION TESTS:

The fermentation of individual sugars was tested out separately. Only the important sugars were put up namely, glucose, lactose, sucrose.

Procedure: The sugar solutions were inoculated from the peptone water with the help of Pasteur pipette and incubated overnight at 37 °C for upto 7 days. The tubes are examined for acid and gas production.

Interpretation:

Pink discolouration of medium: Acid production (fermentation of the sugar)

No colour change No fermentation of the sugar

Gas bubbles in Durham's tube Gas production

OXIDATION-FERMENTATION TEST (Hugh- Leifson's method)

Procedure: Hugh-Leifson's basal medium is prepared and the carbohydrate to be added is sterilized separately and added to give a final concentration of 1%. The medium is then stabbed to a depth of about 4 cm.

Duplicate tubes of medium are inoculated by stabbing. One tube is promptly covered with a layer of sterile melted petroleum jelly to a depth of 5-10 mm and both are incubated for upto 30 days.

Interpretation: Fermenting organisms produce an acid reaction throughout the medium in covered (anaerobic) as well as the open (aerobic) tube.

Oxidizing organisms produce an acid reaction only in the open tube.

Organisms that cannot breakdown the carbohydrate aerobically or anaerobically produce an alkaline reaction in the open tube and no change in covered tube.

NITRATE REDUCTION TEST

Principle: This demonstrates the presence of nitrate reductase enzyme which reduces nitrate into nitrite.

Test organism is incubated in 5ml of nitrate broth for 24 to 48 hours. Equal volumes of reagent A and B are mixed just before use. 0.1 ml of the reagent mixture is added to the culture.

Interpretation: Development of red colour within few minutes is considered positive.

AMINOACID DECARBOXYLATION TEST

Principle: Decarboxylases are a group of substrate-specific enzymes, which are capable of reacting with carboxyl portion of amino acids, forming alkaline-reacting amines, with formation of $C0_2$ as a second product.

Add 10g (final concentration – 1%) of L (levo) form of amino acid (lysine, ornithine or arginine).

Dissolve the solids in water and adjust the pHto 6. Add the indicators, mix and distributed into four equal portions. To this, add the amino acids to be tested except the control tube.

Procedure: From a well isolated colony of the test organism, inoculate two tubes of Moeller decarboxylase medium, one containing the amino acid to be tested, the other to be used as a control tube devoid of amino acid. Overlay both tubes with sterile mineral oil to cover about 1cm of the surface and incubate at 37^0 C for 18-24 hours.

Interpretation: The medium first becomes yellow due to acid production from glucose fermentation; later, if decarboxylation occurs, the medium becomes purple. The control tube remains yellow.

ANNEXURES

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI-3

EC Reg No.ECR/270/Inst./TN/2013
Telephone No. 044 25305301
Fax : 011 25363970

CERTIFICATE OF APPROVAL

To
Dr. R.Krithiga,
Postgraduate M.D.(Microbiology),
Madras Medical College,
Chennai - 600 003.

Dear Dr.R.Krithiga,

The Institutional Ethics Committee has considered your request and approved your study titled **"A study on biofilm formation in organisms causing central venous catheter related blood stream infection in intensive care unit patients in a tertiary care hospital". No.25102014.**

The following members of Ethics Committee were present in the meeting held on 07.10.2014 conducted at Madras Medical College, Chennai-3.

1. Dr.C.Rajendran, M.D.,	: Chairperson
2. Dr.R.Vimala, M.D., Dean, MMC, Ch-3	: Deputy Chairperson
3. Prof.B.Kalaiselvi, M.D., Vice-Principal, MMC, Ch-3	: Member Secretary
4. Prof.R.Nandhini, M.D., Inst.of Pharmacology, MMC	: Member
5. Prof.K.Ramadevi, Director i/c, Inst.of Biochemistry, MMC	: Member
6. Prof.Saraswathy, M.D., Director, Pathology, MMC, Ch-3	: Member
7. Prof.S.G.Sivachidambaram, M.D., Director i/c, Inst.of Internal Medicine, MMC	: Member
8. Thiru S.Rameshkumar, Administrative Officer	: Lay Person
9. Thiru S.Govindasamy, B.A., B.L.,	: Lawyer
10. Tmt.Arnold Saulina, M.A., MSW.,	: Social Scientist

We approve the proposal to be conducted in its presented form.

The Institutional Ethics Committee expects to be informed about the progress of the study and SAE occurring in the course of the study, any changes in the protocol and patients information/informed consent and asks to be provided a copy of the final report.

14/10/14

Member Secretary, Ethics Committee

MEMBER SECRETARY
INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE
CHENNAI-600 003

PROFORMA

- Name
- Age/Sex
- Adress
- Occupation
- Date of Admission
- I.P.no:
- Provisional Diagnosis
- Presenting complaints
- Past History
- Personal History
- General Examination
- Systemic Examination
- Invesigations done
- Systemic Antibiotics given
- Details about Catheterisation

    - ❖ Date of catheterisation
    - ❖ Indication
    - ❖ Type of placement: emergency/elective
    - ❖ No.of attempts to place the catheter
    - ❖ Type of catheter used
    - ❖ Site&side of insertion
    - ❖ Length of catheter inside the patient
    - ❖ Complications during insertion
    - ❖ Frequency of catheter manipulation
    - ❖ Duration of catheterization
    - ❖ Cause of catheter removal
    - ❖ Fever after removal
    - ❖ Length of time in ICU

Microbiological investigation:
Sample collected:

- Swab from exit site
- Catheter tip
- Catheter Blood sample
- Peripheral Blood sample

Isolates obtained :

-Catheter tip: 1) Maki's roll plate

2)Brun Buisson's method

-Catheter blood sample:

-Peripheral blood sample:

Antimicrobial susceptibility pattern:

Biofilm detected:

PATIENT CONSENT FORM

TITLE OF THE STUDY :"A study on Biofilm formation in organisms causing Central Venous Catheter related blood stream infections in intensive care unit patients in a tertiary care hospital"

Name : Date :

Age : OP No :

Sex : Project Patient No :

Documentation of the informed consent

I _____ have read the information in this form (or it has been read to me). I was free to ask any questions and they have been answered. I hereby give my consent to be included as a participant in **"A study on Biofilm formation in organisms causing Central Venous Catheter related blood stream infections in intensive care unit patients in a tertiary care hospital''**

I have read and understood this consent form and the information provided to me.

0I have had the consent document explained to me.

1. I have been explained about the nature of the study.
2. I have been explained about my rights and responsibilities by the investigator.
3. I have informed the investigator of all the treatments I am taking or have taken in the past _____ months including any native (alternative) treatment.

4. I have been advised about the risks associated with my participation in this study.

5. I agree to cooperate with the investigator and I will inform him/her immediately if I suffer unusual symptoms.

6. I have not participated in any research study within the past _____ month(s).

7. I am aware of the fact that I can opt out of the study at any time without having to give my reason and this will not affect my future treatment in this hospital.

8. I am also aware that the investigator may terminate my participation in the study at any time, for any reason, without any consent.

9. I hereby give permission to the investigators to release the information obtained from me as result of participation in this study to the sponsors, regulatory authorities, Govt. agencies, and IEC. I understand that they are publicly presented.

10. I have understood that my identity will be kept confidential if my data are publicly presented.

11. I have had my questions answered to my satisfaction.

12. I have decided to be in the research study.

I am aware that if I have any question during this study, I should contact the investigator. By signing this consent form I attest that the information given in this document has been clearly explained to me and understood by me, I will be given a copy of this consent document.

For participants:

Name and signature / thumb impression of the participant (or legal representative if participant incompetent/For age 10-17 yrs-Name& signature of the parent/guardian.)

Name _____

Signature_____

Date_____

Name and Signature of impartial witness (required for illiterate patients):

Name _____

Signature_____

Date_____

Address and contact number of the impartial witness:

Name and Signature of the investigator or his representative obtaining consent:

Name _____

Signature_____

Date_____

MASTER CHART

KEY TO MASTER CHART

Duration of catheterization

1 - ≤7 days
2 - >7 days

Indication for catheterization

A-Fluid replacement during surgery

B-To resuscitate the patient from shock

C-Lack of peripheral venous access

D-Blood transfusion and Total parenteral nutrition

E-Central Venous Pressure monitoring

F-Dialysis

Duration of ICU stay

1- ≤1week
2- 1 to 2 weeks
3- 2 to 3 weeks
4- 3 to ≥ 4 weeks
5-

Sample Category

Category[1]- Patients with suspected CRBSI with maintenance Central venous catheterization.

Category[2]- Patients with suspected CRBSI in whom maintenance catheterization is not indicated and central venous catheter can be removed

Category[3]- For patients with difficult peripheral vein access.

Id.No	AGE/SEX	ICU	DURATION OF STAY	INDICATION FOR CATHETER	ELECTIVE/ EMERGENCY	SITE OF CATHETER	DURATION OF CATHETER	FREUENCY OF CATHETER MANIPULATIONS	CLINICAL DIAGNOSIS	SAMPLE CATEGORY	GROWTH	AMIKACIN	GENTAMYCIN	CIPROFLOXACIN	ERYTHROMYCIN	CZ	CZC	COTRIMOXAZOLE	TETRA	IMIPENEM	PT	PEN	CTX	ctc	CX /VMIC	VORU/FLUCANOZOLE	BIOFILM TUBE	BIOFILM CRA	BIOFILM MC	OUTCOME
1	48 /M	SICU	2	B	EMERGE	RT.SUBCLAVIAN	1	2	HOLLOW VISCOUS PERFORATION	1																				
2	55/m	IMCU	1	D	ELECTIVE	RT.JUGULAR	1	2	MYASTHENIA GRAVIS	1																				
3	28/F	IMCU	2	F	ELECTIVE	LT.JUGULAR	2	2	APLASTIC ANEMIA	2																				
4	43/M	IMCU	1	F	EMERGENCY	LT.SUBCLAVIAN	1	3	ACUTE PANCREATITIS	1																				
5	40/M	IMCU	3	D	EMERGENCY	RT.FEMORAL	2	2	GBS	2																				
6	55/M	SICU	1	B	EMERGENCY	LT.SUBCLAVIAN	1	3	RTA BLUNT INJURT ABDOMEN	1	ACINETOBACTER BAUMANII	R	R	R		R	R	S	R	S	S						M	ST	ST	SUCCUMBED
7	35/M	ITCU	4	F	EMERGENCY	RT.SUBCLAVIAN	1	2	HEAD INJURY	2																				
8	38/M	SICU	1	F	EMERGENCY	RT.SUBCLAVIAN	1	3	ACUTE PANCREATITIS	1																				
9	68/F	IMCU	2	C	EMERGENCY	LT.FEMORAL	2	3	CVA	3																				
10	58/F	SICU	1	A	ELECTIVE	RT.JUGULAR	1	2	CA BREAST	1																				
11	52/M	IMCU	4	C	EMERGENCY	RT.FEMORAL	2	3	COPD/TYPE 2 RENAL FAILURE	3	PSEUDOMONAS AERUGINOSA	S	S	S		S	S			S	S						W	M	W	RECOVERED
12	32/M	IMCU	4	D	EMERGENCY	LT.SUBCLAVIAN	2	3	HANGING/HEAD INJURY	1																				
13	46/M	ITCU	3	B	EMERGENCY	SUBCLAVIAN	2	3	RTA POLYTRAUMA	2			.																	
14	36/M	SICU	2	B	EMERGENCY	JUGULAR	2	2	SELF FALL POPLITEAL VEIN INJURY	1																				
15	29/F	IMCU	2	C	ELECTIVE	SUBCLAVIAN	1	2	POST NATAL PPH /ARF	3																				
16	67/F	SICU	1	A	ELECTIVE	SUBCLAVIAN	1	2	INTESTINAL OBSTRUCTION	1																				
17	28/F	IMCU	3	D	ELECTIVE	RT.SUBCLAVIAN	2	4	OPC POISONING	2	KLEBSIELLA PNEUMONIAE	S	S	R				R	S	S	S		R(ESBL)	S(ESBL)			ST	ST	ST	RECOVERED
18	48/F	SICU	1	A	ELECTIVE	JUGULAR	1	2	MNG	1																				
19	22/M	SICU	2	F	ELECTIVE	LT.FEMORAL	1	3	CHONDROMYXOID FIBROMA	2																				
20	35/M	IMCU	2	F	EMERGENCY	SUBCLAVIAN	2	2	APLASTIC ANEMIA	1																				
21	49/M	ITCU	4	C	EMERGENCY	JUGULAR	1	2	HEAD INJURY	3																				
22	55/M	ITCU	3	B	EMERGENCY	RT.FEMORAL	2	3	RTA POLYTRAUMA	1																				
23	58/M	ITCU	2	B	EMERGENCY	LT.JUGULAR	2	2	POLYTRAUMA VASCULAR INJURY	3	STAPHYLOCOCCUS AUREUS	S		S	R			S	S			R			S		W	W	W	RECOVERED
24	25/F	IMCU	2	C	EMERGENCY	SUBCLAVIAN	1	3	POST LSCS/ AKI	3																				

Id.No	AGE/SEX	ICU	DURATION OF STAY	INDICATION FOR CATHETER	ELECTIVE/ EMERGENCY	SITE OF CATHETER	DURATION OF CATHETER	FREUENCY OF CATHETER MANIPULATIONS	CLINICAL DIAGNOSIS	SAMPLE CATEGORY	GROWTH	AMIKACIN	GENTAMYCIN	CIPROFLOXACIN	ERYTHROMYCIN	CZ	CZC	COTRIMOXAZOLE	TETRA	IMIPENEM	PT	PEN	CTX	ctc	CX /VMIC	VORU/FLUCANOZOLE	BIOFILM TUBE	BIOFILM CRA	BIOFILM MC	OUTCOME
25	45/M	SICU	1	F	ELECTIVE	JUGULAR	1	2	RCC	2																				
26	53/M	SICU	2	B	EMERGENCY	SUBCLAVIAN	2	3	ILEAL PERFORATION	1																				
27	29/M	ITCU	3	D	EMERGENCY	JUGULAR	2	2	SOL HYDROCEPHALUS/SHUN	2																				
28	26/F	IMCU	4	D	EMERGENCY	LT.FEMORAL	2	3	RESPIRATORY FAILURE	2																				
29	34/M	SICU	2	A	ELECTIVE	SUBCLAVIAN	1	2	POST APPENDECTOMY WITH INTESTINAL	1																				
30	20/F	IMCU	3	F	EMERGENCY	RT.JUGULAR	2	2	ACUTE INTERMITTENT PORPHYRIA	1	PSEUDOMONAS AERUGINOSA	R	R	R		R	S			S	S						W	W	W	RECOVERED
31	36/F	SICU	1	A	ELECTIVE	SUBCLAVIAN	1	2	POST CHOLECYSTECTOMY	1																				
32	49/F	SICU	1	A	ELECTIVE	LT.FEMORAL	1	2	INCISIONAL HERNIA	2																				
33	50/F	IMCU	3	C	EMERGENCY	RT.FEMORAL	2	2	AORTIC DISSECTION	3																				
34	51/M	IMCU	2	D	EMERGENCY	RT.SUBCLAVIAN	1	2	DCLD/HEPATIC ENCEPHALO[ATHY GR 2	1	KLEBSIELLA OXYTOCA	S	S	R				R	S	S	S		R	S(ESBL)			M	S	S	RECOVERED
35	66/M	SICU	3	F	ELECTIVE	JUGULAR	2	3	INTESTINAL OBSTRUCTION	1																				
36	45/M	ITCU	4	B	EMERGENCY	SUBCLAVIAN	1	2	RTA POLYTRAUMA	2																				
37	52/F	SICU	2	C	ELECTIVE	SUBCLAVIAN	2	3	CA BREAST	3																				
38	43/M	IMCU	3	D	EMERGENCY	RT.FEMORAL	2	2	GBS	1																				
39	39/M	SICU	3	B	EMERGENCY	JUGULAR	2	3	SMALL BOWEL GANGRENE	2																				
40	27/M	IMCU	4	F	EMERGENCY	RT.FEMORAL	2	4	ACUTE MENINGO ENCEPHALITIS	2	PROTEUS VULGARIS	S	R	R				R	S	S	S		R	(ESBL)			N	W	W	RECOVERED
41	35/M	ITCU	3	A	EMERGENCY	SUBCLAVIAN	1	3	HEAD INJURY	1																				
42	48/F	SICU	2	F	ELECTIVE	JUGULAR	1	3	CA CERVIX	1																				
43	72/F	IMCU	2	F	ELECTIVE	SUBCLAVIAN	1	2	CVA	1																				
44	70/M	SICU	3	C	EMERGENCY	JUGULAR	2	3	CA RECTUM	3																				
45	35/M	SICU	1	A	EMERGENCY	SUBCLAVIAN	1	2	SIGMOID VOLVULUS	2																				
46	74/M	ITCU	3	C	ELECTIVE	RT.JUGULAR	2	2	SELF FALL HEAD INJUR	2	PSEUDOMONAS AERUGINOSA	S	R	R		R	R			S	S						ST	ST	ST	SUCCUMBED
47	56/M	SICU	1	C	ELECTIVE	SUBCLAVIAN	1	2	HODGKINS DISEASE	3																				
48	35/F	SICU	2	A	ELECTIVE	LT.FEMORAL	1	3	EMPYEMA GB	1																				

Id.No	AGE/SEX	ICU	DURATION OF STAY	INDICATION FOR CATHETER	ELECTIVE/ EMERGENCY	SITE OF CATHETER	DURATION OF CATHETER	FREUENCY OF CATHETER MANIPULATIONS	CLINICAL DIAGNOSIS	SAMPLE CATEGORY	GROWTH	AMIKACIN	GENTAMYCIN	CIPROFLOXACIN	ERYTHROMYCIN	CZ	CZC	COTRIMOXAZOLE	TETRA	IMIPENEM	PT	PEN	CTX	ctc	CX /VMIC	VORI/FLUCANOZOLE	BIOFILM TUBE	BIOFILM CRA	BIOFILM MC	OUTCOME
49	65/F	IMCU	1	D	EMERGENCY	SUBCLAVIAN	1	3	HEPATIC ENCEPHALOPATHY	2																				
50	29/M	IMCU	2	E	EMERGENCY	JUGULAR	2	3	SNAKE BITE	1																				
51	69/M	SICU	2	C	ELECTIVE	RT.FEMORAL	1	3	MALIGNANT OBSTRUCTIVE JAUNDICE	3																				
52	56/M	ITCU	3	B	EMERGENCY	SUBCLAVIAN	2	3	RTA POLYTRAUMA	1																				
53	45/F	ITCU	4	F	EMERGENCY	RT.SUBCLAVIAN	2	3	RTA POLYTRAUMA	1	STAPHYLOCOCCUS AUREUS	S	R	S	R			R	S			R	MRSA				M	ST	ST	RECOVERED
54	26/M	IMCU	1	C	EMERGENCY	JUGULAR	1	3	PARAQUAT POISONING	3																				
55	49/F	SICU	1	A	ELECTIVE	SUBCLAVIAN	1	3	CA TONGUE	1																				
56	36/F	ITCU	2	C	EMERGENCY	LT.FEMORAL	1	2	RT. CEREBELLAR SOL / HYDROCEPH	3																				
57	48/M	ITCU	3	B	EMERGENCY	JUGULAR	2	3	RTA POLYTRAUMA	2																				
58	65/M	SICU	2	F	ELECTIVE	SUBCLAVIAN	1	2	INTESTINAL OBSTRUCTION	1																				
59	55/M	SICU	2	F	EMERGENCY	RT.SUBCLAVIAN	2	3	INTESTINAL OBSTRUCTION	1	KLEBSIELLA PNEUMONIAE	S	R	S				S		S	S		R(ESBL)	S(ESBL)			M	ST	ST	RECOVERED
60	45/M	IMCU	1	D	ELECTIVE	SUBCLAVIAN	1	2	HODGKINS DISEASE	2																				
61	37/F	IMCU	2	E	ELECTIVE	SUBCLAVIAN	2	2	SLE/CKD	1																				
62	38/M	ITCU	3	C	EMERGENCY	FEMORAL	2	3	RTA POLYTRAUMA	3																				
63	46/F	SICU	2	A	ELECTIVE	JUGULAR	1	2	SPLENECTOMY	2																				
64	74/M	IMCU	2	B	EMERGENCY	LT.JUGULAR	2	2	SHT/CVA/MODS	1	STAPHYLOCOCCUS EPIDERMIDIS	S	R	S	R			S	S			R			S		NEGATIVE	W	W	RECOVERED
65	59/M	SICU	3	A	EMERGENCY	SUBCLAVIAN	2	3	SMALL BOWEL GANGRENE	1																				
66	32/M	ITCU	4	C	EMERGENCY	JUGULAR	1	2	RTA HEAD INJURY	3																				
67	29/M	IMCU	3	F	EMERGENCY	FEMORAL	1	3	ACUTE MENINGO ENCEPHALITIS	1																				
68	46/M	SICU	2	B	EMERGENCY	SUBCLAVIAN	2	3	RTA BLUNT INJURT ABDOMEN	1																				
69	52/F	SICU	2	A	ELECTIVE	SUBCLAVIAN	2	2	CA BREAST	2																				
70	21/F	IMCU	4	C	ELECTIVE	FEMORAL	2	2	KRABBE LEUKODYSTROPHY	3																				
71	62/M	SICU	1	A	EMERGENCY	RT.SUBCLAVIAN	2	3	HOLLOW VISCOUS PERFORATION	1	ACINETOBACTER BAUMANII	S	R	R		R	S	S	S	S	S						M	S	S	RECOVERED

Id.No	AGE/SEX	ICU	DURATION OF STAY	INDICATION FOR CATHETER	ELECTIVE/ EMERGENCY	SITE OF CATHETER	DURATION OF CATHETER	FREUENCY OF CATHETER MANIPULATIONS	CLINICAL DIAGNOSIS	SAMPLE CATEGORY	GROWTH	AMIKACIN	GENTAMYCIN	CIPROFLOXACIN	ERYTHROMYCIN	CZ	CZC	COTRIMOXAZOLE	TETRA	IMIPENEM	PT	PEN	CTX	ctc	CX /VMIC	VORU/FLUCANOZOLE	BIOFILM TUBE	BIOFILM CRA	BIOFILM MC	OUTCOME
72	59/M	ITCU	3	B	EMERGENCY	SUBCLAVIAN	2	2	RTA POLYTRAUMA	2																				
73	54/F	SICU	1	A	ELECTIVE	JUGULAR	1	2	MNG	1																				
74	60/M	SICU	1	D	EMERGENCY	SUBCLAVIAN	1	2	MALIGNANT OBSTRUCTIVE JAUNDICE	2																				
75	65/M	IMCU	1	D	EMERGENCY	FEMORAL	1	3	COPD/ COR PULMONALE	2																				
76	59/M	IMCU	2	C	EMERGENCY	JUGULAR	2	2	SLEEP APNEA /RESP. FAILURE	3																				
77	66/F	ITCU	3	F	ELECTIVE	SUBCLAVIAN	2	2	SELF FALL HEAD INJUR	1																				
78	45/M	SICU	2	C	EMERGENCY	SUBCLAVIAN	2	3	HOLLOW VISCOUS PERFORATION	3																				
79	48/F	ITCU	3	C	EMERGENCY	JUGULAR	2	2	CRUSH INJURY BOTH LIMBS	3																				
80	39/M	SICU	1	A	EMERGENCY	RT.FEMORAL	1	3	HOLLOW VISCOUS PERFORATION	1	KLEBSIELLA PNEUMONIAE	R	R	R				R		S	R		R	S(ESBL)			W	R	M	RECOVERED
81	29/M	IMCU	2	D	EMERGENCY	SUBCLAVIAN	1	3	OPC POISONING	2																				
82	33/M	IMCU	2	E	EMERGENCY	SUBCLAVIAN	2	2	SNAKE BITE	2																				
83	21/F	SICU	1	A	ELECTIVE	JUGULAR	1	3	SLE/VULVAL ULCERATION	1																				
84	23/M	ITCU	3	F	ELECTIVE	FEMORAL	1	2	RT CEREBRAL SOL	2																				
85	34/F	SICU	1	B	EMERGENCY	SUBCLAVIAN	1	3	APPENDICULAR PERFORATION	1																				
86	42/M	SICU	1	C	EMERGENCY	FEMORAL	1	2	HOLLOW VISCOUS PERFORATION	3																				
87	45/M	ITCU	2	C	EMERGENCY	RT.FEMORAL	1	2	RTA HEAD INJURY	3	CANDIDA ALBICANS															S	N	W	W	RECOVERED
88	58/M	ITCU	4	F	ELECTIVE	SUBCLAVIAN	2	2	SOL HYDROCEPHALUS/SHUN	2																				
89	48/M	SICU	3	C	EMERGENCY	SUBCLAVIAN	2	3	STAB INJURY ABDOMEN	3																				
90	26/F	IMCU	1	E	EMERGENCY	SUBCLAVIAN	1	2	POST LSCS/ AKI	1																				
91	72/M	IMCU	1	F	ELECTIVE	FEMORAL	1	2	CVA	2																				
92	66/M	SICU	2	A	EMERGENCY	SUBCLAVIAN	2	3	SPLENECTOMY	2																				
93	73/F	ITCU	2	C	EMERGENCY	JUGULAR	1	2	SELF FALL	3																				
94	49/M	ITCU	4	F	EMERGENCY	SUBCLAVIAN	2	2	ICSOL/ HYDROCEPHALUS	2																				
95	36/M	SICU	2	C	EMERGENCY	LT.JUGULAR	2	3	ILEAL PERFORATION	2	STAPHYLOCOCCUS AUREUS	S	S	R	S			S	R			R	MRSA		R		ST	ST	ST	RECOVERED

Id.No	AGE/SEX	ICU	DURATION OF STAY	INDICATION FOR CATHETER	ELECTIVE/ EMERGENCY	SITE OF CATHETER	DURATION OF CATHETER	FREUENCY OF CATHETER MANIPULATIONS	CLINICAL DIAGNOSIS	SAMPLE CATEGORY	GROWTH	AMIKACIN	GENTAMYCIN	CIPROFLOXACIN	ERYTHROMYCIN	CZ	CZC	COTRIMOXAZOLE	TETRA	IMIPENEM	PT	PEN	CTX	ctc	CX /VMIC	VORU/FLUCANOZOLE	BIOFILM TUBE	BIOFILM CRA	BIOFILM MC	OUTCOME
96	42/M	SICU	3	B	EMERGENCY	SUBCLAVIAN	2	2	ILEAL PERFORATION	2																				
97	35/F	ITCU	3	F	EMERGENCY	FEMORAL	2	2	HEAD INJURY	1																				
98	49/M	ITCU	4	C	EMERGENCY	SUBCLAVIAN	1	2	RTA POLYTRAUMA	3																				
99	68/M	SICU	1	A	ELECTIVE	SUBCLAVIAN	1	3	INTESTINAL OBSTRUCTION	2																				
100	35/F	IMCU	1	C	ELECTIVE	FEMORAL	1	2	HYPOTHYROIDISM/ANASARCA	3																				
101	38/F	IMCU	3	E	ELECTIVE	SUBCLAVIAN	2	2	SLE/ LUPUS NEPHRITIS	1																				
102	75/M	SICU	2	F	ELECTIVE	JUGULAR	2	2	CA RECTUM	2																				
103	40/M	SICU	3	A	ELECTIVE	RT.SUBCLAVIAN	2	2	INTESTINAL OBSTRUCTION	2	PSEUDOMONAS AERUGINOSA	S	R	R		R	R			S	S						W	M	M	RECOVERED
104	46/M	SICU	2	B	EMERGENCY	JUGULAR	2	3	HOLLOW VISCOUS PERFORATION	2																				
105	33/F	IMCU	2	C	EMERGENCY	SUBCLAVIAN	2	3	GDM/HYPOKALEMIA	3																				